Microbiology

PA-01

台南市立醫院
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Staphylococcus haemolyticus endocarditis: a case report

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Introduction

The case numbers of Staphylococcus *haemolyticus* endocarditis were rare in the literature. Herein, we reported a case

Case report

A 90–year–old male patient with uremia received hemodialysis treatment was admitted due to fever on 20 Apriil 2014. After admission, piperacillin–tazobactam was given for treatment. On 23 April, transthoracic heart echo revealed a vegetation with a size of 2.5 cm ×1.83 cm located on the mitral valve. On 24 April, the two sets of blood culture taken on 20 April were positive with *S. haemolyticus*, identified by Phoenix automachine (Becton Dickinson and Company, Sparks, MD). Antimicrobial susceptibility testing done by Phoenix automachine revealed the two isolates were resistant to oxacillin with minimal inhibitory concentration (MIC) 0.5 mg/L, and susceptible to clindamycin with MIC≦0.5 mg/L , fusidic acid with MIC≦0.5 mg/L, linezolid with MIC≦1 mg/L, and vancomycin with MIC≦1 mg/L. Consequently, *S. haemolyticus* endocartitis was definitively diagnosed according to modified Duke criteria, and then vancomycin was given for treatment. On the same day, another two sets of blood culture were taken again. Thereafter, fever still persisted despite treatment with vancomycin. On 28 April, the two sets of blood culture taken on 24 April were also positive with *S. haemolyticus*, and the results of antimicrobial susceptibility testing were the same as previous isolates. Because the patient’ s condition was gone downhill persistently, valve replacement was suggested; however, the family refused this operation. Finally, the patient expired on 5 May 2015.

Discussion

Once blood culture is positive with coagulase–negative staphylococci (CoNS), especially more than two sets of blood culture positive with CoNS, the possibility of bacterial endocarditis should be kept in mind. In this setting, heart echo, especially transesophageal heart echo, may be performed to diagnose bacterial endocarditis.

Microbiology

PA-02

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A Rare Case of *Tsukamurella paurometabola* Infection in Immunocompromised Patient

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Introduction

Catheter-related bloodstream infections (CR-BSI) are important complications in patients with long-term indwelling central venous catheters.We report a rare cases of bacteremia caused by *Tsukamurella paurometabola*, A 60 year-old female with history of hypertension and gastrointestinal stromal tumor, Besides, ischemic bowel disease because of total occlusion of superior mesenteric artery was noted. The emergent operation with jejunostomy was performed on Sep. 24th, 2014. She received glivec for gastrointestinal stromal tumor and peripherally inserted central catheter with total parenteral nutrition supplement for her short bowel. In mid-September 2015, she had frequent fever and chills, which was followed by dizziness and general weakness. The first blood culture results reveal gram positive bacilli which was suspected as skin contamination .However , she continue had fever and chills .The 2nd blood culture were performed after 5 days. During hospitalization with serial examination, blood culture revealed Gram positive bacilli (Figure 1) only and catheter related infection was impressed. However, blood culture revealed Gram positive bacilli again, and a total of 8 sets were reported since last hospitalization.

Materials and Methods

We inoculated a positive blood culture onto BAP, Chocolate and EMB agar in three culture flasks following guideline for 24-hour subculture. Afterwards, the strain first underwent analyses using traditional methods, including Gram stain, acid-fast stain, oxidase and catalase biochemical tests. It was then identified and analyzed using the bioMérieux VITEK II system and the BD Crystal Rapid Gram-Positive ID Kit for matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) (including VITEK MS and Bruker MALDI- TOF MS). Finally, 16S ribosomal RNA gene sequencing was conducted using the forward primer E8FE9 and the reverse primer U1510R and sequence alignments were produced and analyzed to verify this strain.

Results

The test results using the traditional methods are summarized in the schedule(Table1.). The analysis results using the bioMérieux VITEK II indicate *Granulicatella elegans* while those using the BD Crystal Rapid Gram-Positive ID Kit point to *Brevibacillus brevis*. The aforementioned two identified species can be eliminated because their biochemical reactions do not match the results using the traditional methods. In contrast, 16S ribosomal RNA gene sequencing validates that this strain is *Tsukamurella paurometabola* and all of its biochemical reactions match the results of the traditional tests we conducted.

Tsukamurella paurometabola are aerobic gram-positive organisms from the order *Actinomycetales*. It is a, non–spore-forming, partially acid-fast bacilli(Figure 2.), gram-positive bacillus that does not produce aerial hyphae. It grows best at temperatures of 37 ˚C,and it forms flat colonies that appear dry , may have rhizoid edges, and are tan to gray in color (Figure 4). After prolonged incubation, the colonies have a rough appearance (Figure 3) .

Traditional test		Auto identification system	
Test Methods	Result	Test Methods	Result
Urease	Positive	BioMérieux VITEK II	*Granulicatella elegans*
MR-VP Test	Negative	BD Crystal Rapid Gram-Positive ID Kit	*Brevibacillus brevis*
Indole Test	Negative		
Esculin iron	Positive	VITEK MS	No identification
6.5% Nacl	Negative	Bruker MALDI-TOF	No identification
Bile esculin	Positive		
Oxidase	Negative	16S rRNA Sequencing	*Tsukamurella paurometabola*
Catalase	Weak positive		

Table 1.The result of biochemical tests and automated identification

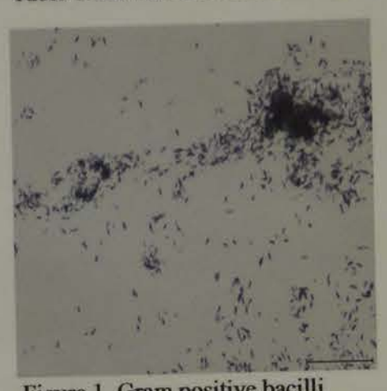


Figure 1. Gram positive bacilli

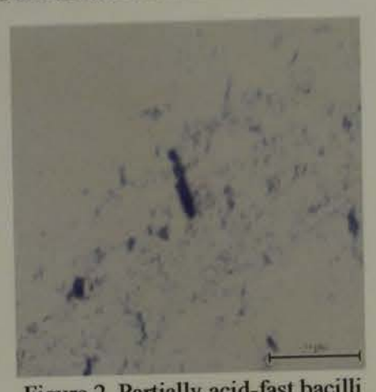


Figure 2. Partially acid-fast bacilli



Figure 3. Growth on Chocolate agar



Figure 4. Growth on BAP

Discussion

We reported a rare bloodstream infection caused by that *Tsukamurella paurometabola* can cause bloodstream infection, making real-time strain identification of utter importance. Given that there is no CLSI Guidline for treating this pathogen infection and the potential role of *Tsukamurella paurometabola* in BSI. Further research is necessary and will benefit physicians treating this infection.

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Microbiology

PA-04

台南市立醫院
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Nosocomial Enterobacter cloacae endocarditis: a case report

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Introduction

The case numbers of Enterobacter cloacae endocarditis were rare in the literature. Herein, we reported a case, the first case of *E. cloacae* and even gram–negative bacilli endocarditis in this hospital.

Case report

A 47–year–old male patient was admitted under impression of diabetic ketoacidosis on 21 November 2015. He had underdying diseases of liver cirrhosis, hypertension, and diabetes. After admission, only conservative therapy, including subcutaneous insulin injection, was given. On 27 November, nosocomial fever occurred, and then vancomycin and ceftazidime were given as empiric antibiotics. Thereafter, fever had decreased gradually and subsided on two days later. On the same day, a transthoracic heart echo was performed and revealed calcified aortic valve (AV) with mild aortic stenosis (AS) and aortic regurgitation (AR) as well as highly suspected vegetations on AV. On 28 November, a transesophageal heart echo was performed and revealed multiple vegetations on AV with mild AS and AR. On 30 November, all four blood cultures taken on 27 November, the time of blood culture drawn was 13:40, 14:10, 17:20, and 17:59, respectively, were positive with *E. cloacae*. All four *E. cloacae* isolates had the same antimicrobial susceptibility testing results, including resistance to cefazolin, cefuroxime, and amoxicillin–clavulanate as well as susceptibility to ceftriaxone, ceftazidime, piperacillin–tazobactam, gentamcin, amikacin, levofloxacin, trimethoprin–sulmethoazole, and tigecycline. Consequently, nosocomial *E. cloacae* endocarditis was diagnosed according to the modified Duke crteria, and then doripenem was given for definitive antibiotic treatment. On 24 December, a 28–day treatment course was completed, and the patient was discharged.

Discussion

According to the modified Duke criteria, persistently positive blood cultures with first and last blood culture taken at least one hour apart are one major criteria to diagnose infectious endocarditis. Herein, we suggest heart echo should be performed to diagnose infectious endocarditis once encountering persistently positive blood cultures with gram–negative bacilli.

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PA-06

From 3 May to 30 June 2016, 3
been exposed to 0.9% saline
collected at the hospital were fo
to investigate the relatedness o
field gel electrophoresis (PFGE
patients and the isolates from s
PFGE.

- **Patients and isolates.** *Ral*
between 3 May to 30 June
vials of 0.9% sodium chlori
- **Microbiological character**
susceptibility testing was pe
- **PFGE** isolates were digest
with a CHEF-DR II appara
ntepreted according to sta
- **Dendrogram Analysis by**
protein mass data.

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Figure 1 a,b Pulsed-field g
14093; C2, BCR

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- The PFGE results reve
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- We also used the Vite
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a new way to analyze

Microbiology

PA-05

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Rhodotorula in blood culture: significance or non- significance

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Introduction

The past few decades have witnessed infections due to yeasts as a common and significant cause of morbidity and mortality in immunocompromised patients. Previously fungal infections were caused by yeasts like *Candida* spp. *Cryptococcus neoformans* and dimorphic fungi. However, currently saprophytic yeasts like *Trichosporon* spp., *Malassezia* spp., *Geotrichum candidum* and *Rhodotorula* spp. are emerging pathogens.

Rhodotorula species infection, which was mainly presumed as contamination or colonization of specimen, is much less common than other yeast. However, the number of this infection has clearly increased during the last few years.

Rhodotorula is an ubiquotous environmental and air borne saprophyte, but now it is being increasingly isolated from immunocompromised patients. Among these infective *Rhodotorula* species, *Rhodotorula mucilaginosa* is the most common strain. And immunocompromised patient, central vein catheter or foreign body implantation are the predominant risk factor of *Rhodotorula mucilaginosa* infection.

Large numbers of studies have described *Rhodotorula* spp. as a cause of human mycoses. Herein, we report two patients whose blood culture grew *Rhodotorula mucilaginosa*

Case Report - 1

The first patient is a 38-year-old male with obstructive sleep apnea under CPAP use and asthma. He presented with fever, cough to ER for a few days with leukocytosis (12900/uL) and neutrophil predominant (94.2%). Meanwhile, *R. mucilaginosa* was cultured in one of the two isolated blood bottles at ER. (Figure 1 and Figure 2) No antifungal regimen was given and his symptom relieved in the follow-up of out patient department three days later.



Figure1 The morphology of *R. mucilaginosa* on Sabouraud Dextrose Agar (SDA)



Figure 2 The microscopic view of *R. mucilaginosa*

Case Report - 2

The second patient is an 80-year-old bedridden male due to old CVA with CAD status post CABG and ESRD under hemodialysis. He presented to out-patient department with right upper chest swelling for 1 week and was referred to ER later. Leukocytosis (29400/uL) was noted but there was no left shift (Neutrophil: 73.0 %). The enlarging tumor was diagnosed as necrotizing fasciitis by image (Figure 3) and he received debridement + fasciectomy twice and under Tygacil + Tapimycin use till the end.

There were only negative finding in blood culture on admission day 1 and contamination in wound culture during admission day 1 and day 9. During admission, there was no significant alteration of neutrophil percentage and WBC count remained higher than 10000/uL. On admission day 18, the patient presented with septic shock with no new-onset fever. And he expired on the next day under using the previous antibiotics. Nevertheless, two sets of blood culture from central vein catheter were performed on the day before the patient expired. One of them grew nothing while the other grew *R. mucilaginosa*. No CVC tip culture was done then.



Figure 3 Right chest mass of patient 2

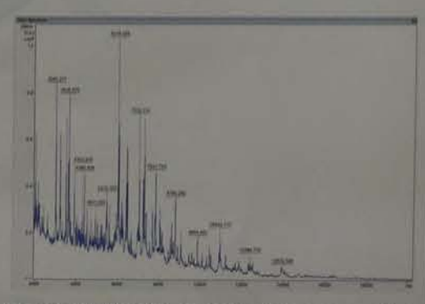


Figure 4 The *R. mucilaginosa* spectra

Method

Sample preparation for MALDI-TOF MS & analysis.
Fresh colony material was smeared on a polished steel MSP 96 target (Bruker Daltonik) using a toothpick. For the direct transfer-formic acid method, 1 µl of 70% formic acid was added to the bacterial spot and allowed to air. Then, overlaid with 1 µl of a saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonik), and air dried at room temperature. The acquisition and analysis of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonik) using the MALDI Biotyper software package(Figure 4). The Bruker bacterial test standard was used for calibration according to the instructions of the manufacturer.

Discussion

Rhodotorula spp. is ubiquitous in distribution. It is found in soil, air, lakes, ocean, water and dairy products. Furthermore, it is non-pathogenic coloniser of plants, humans and other mammals (like shrimps).

While the culture of *Rhodotorula* species from a sterile site, such as blood, is usually indicative of infection and central vein catheter is a common risk factor . But in these cases we should suspect contamination of Rhodotorula during the culture, when it is not suggestive of infection. Which the *R. mucilaginosa* might come from the environment at emergency department in patient 1 and central vein catheter in patient 2. However, we should always keep in mind that and non-bacterial pathogen infection.

Several publications suggested overall mortality rate of 12.6-15% due to Rhodotorula infection. Amphotericin B is the drug of choice in Rhodotorula infection in immunocompromised patients. However, in the immunocompetent patients, this infection can be treated successfully by recovery from underlying conditions and catheter removal

Microbiology

PA-06

A Nosocomial Outbreak of *Ralstonia pickettii* by Contaminated Saline Solution in a Taipei Hospital

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Introduction

From 3 May to 30 June 2015, 31 patients in a hospital in Taipei were culture-positive for *Ralstonia pickettii* from their blood specimens. They had been exposed to 0.9% saline solution which was manufactured by one company (YF Chemical Corp). Two of the 17 saline solution samples collected at the hospital were found to contain *Ralstonia pickettii*. The isolates were analyzed by MALDI-TOF MS for microbial identification. In order to investigate the relatedness of the clinical isolates and the isolates from saline solution, all isolates were analyzed genotypically related by pulsed-field gel electrophoresis (PFGE) analysis. The PFGE (DNA-digested with *SpeI* restriction endonuclease) result showed that the *R.pickettii* from patients and the isolates from saline solution were related. And we also used Vitek MS RUO mode to analyze all isolates, the result was similar with PFGE.

Method & Material

- **Patients and isolates.** *Ralstonia pickettii* isolates from 31 patients of Taipei Veterans General Hospital (a 3000 bed Medical Center), isolated between 3 May to 30 June 2015, were included in this study, including 25 blood samples and 6 port-A flush. Two isolates from two unopened vials of 0.9% sodium chloride solution (the same lot number) belong to two different nursing station.
- **Microbiological characterization** All *Ralstonia pickettii* clinical isolates were identified by MALDI-TOF MS (bioMerieux). Antimicrobial susceptibility testing was performed on all clinical isolates by VITEK 2 (bioMérieux, Marcy l'Étoile, France).
- **PFGE** Isolates were digested with *SpeI* restriction endonuclease according to the manufacturer's directions, and PFGE criteria was carried out with a CHEF-DR II apparatus (Bio Rad Laboratiories, Hercules, CA) at 14°C for 20h at 200 V, with a pulse-time ramp of 5-70 s. Gel were intepreted according to standard criteria.
- **Dendrogram Analysis by MALDI-TOF MS** A research use only (RUO) mode using the SARAMIS™ was used to acquire and process the protein mass data.

Results

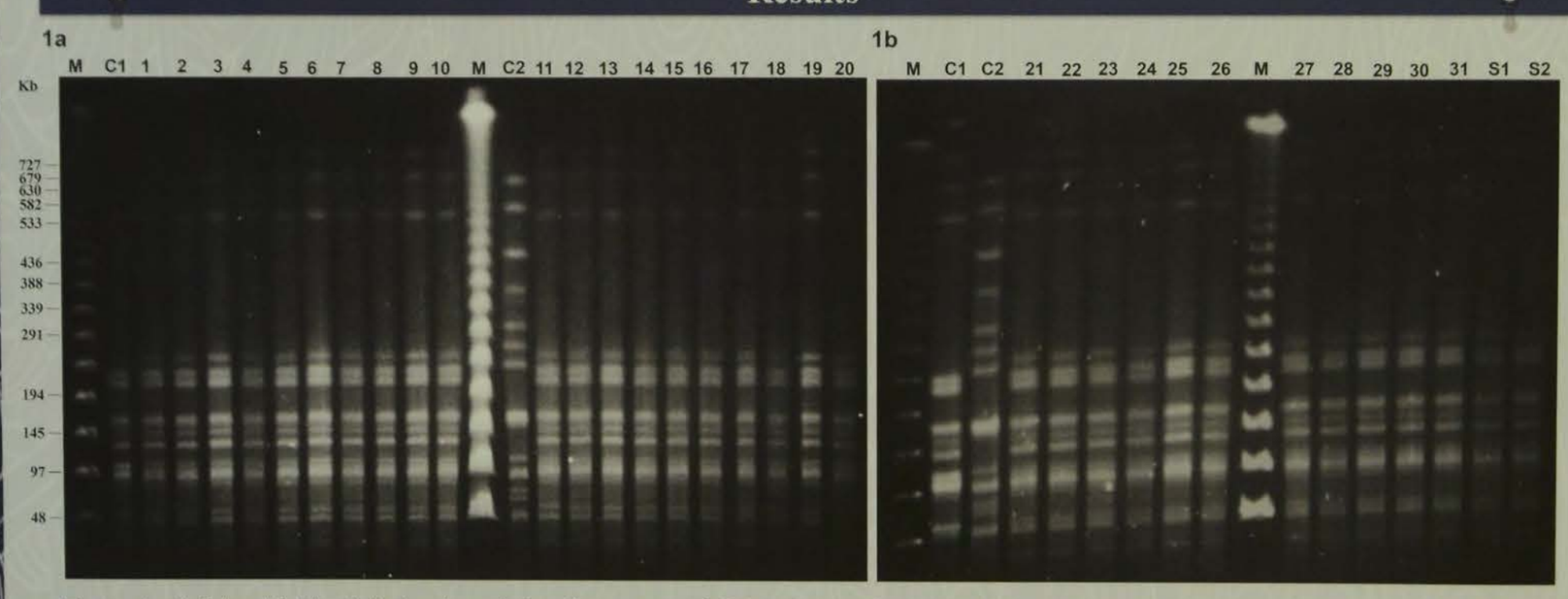


Figure 1 a,b Pulsed-field gel electrophoresis banding pattern of *Ralstonia pickettii*, DNA-digested with *SpeI*. ***M***, molecular size marker. ***C1***, *BCRC 14093*; ***C2***, *BCRC 14820*; ***1~31***, *R. pickettii* isolates from patients at hospital; ***S1,S2***, *R. pickettii* isolates from saline solution.

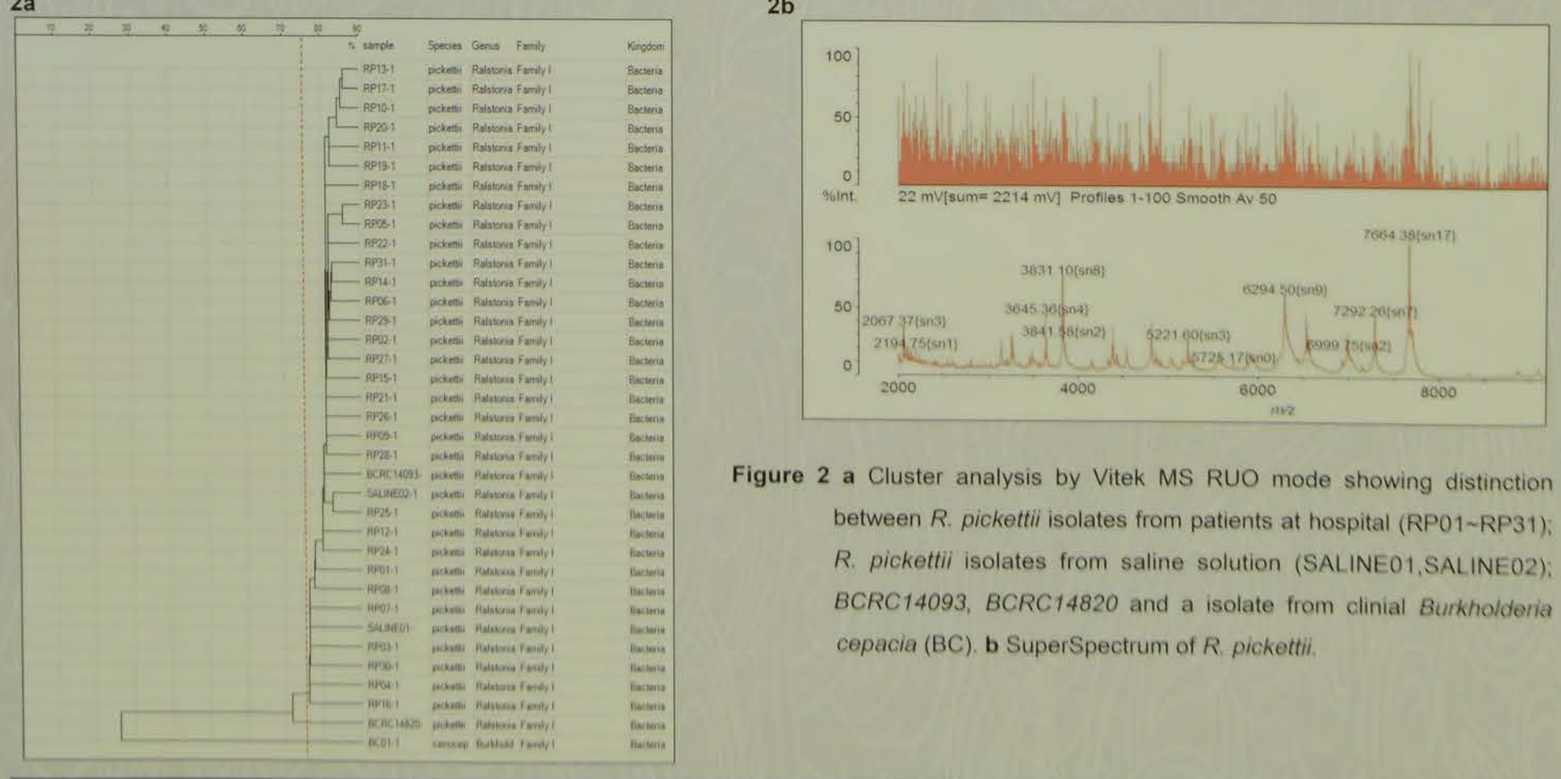


Figure 2 a Cluster analysis by Vitek MS RUO mode showing distinction between *R. pickettii* isolates from patients at hospital (RP01~RP31); *R. pickettii* isolates from saline solution (SALINE01,SALINE02); *BCRC14093*, *BCRC14820* and a isolate from clinial *Burkholderia cepacia* (BC). **b** SuperSpectrum of *R. pickettii*.

Conclusion

- *R. pickettii* has a long history of causing nosocomial outbreaks associated with contaminated solutions used for patient care.
- The PFGE results revealed the isolates from 31 patients and from saline were indistinguishable — they had the same pattern, and the standard strain *BCRC14093* was closely related with them, but *BCRC14820* showed different pattern.
- According to the results of PFGE, we can presume the isolate of 0.9% saline solution and the isolates from patients were clonally related, which meant the outbreak of this infection was caused by the saline solution.
- We also used the Vitek MS RUO mode to analyze the same samples, and it showed a similar result the result. Moreover we can set a intra-species similarity cut-off : 75% (the similarity of strain over 75% represented closely related).
- PFGE needs expensive instrumentation, time consuming (2~3 days) and requires high level skills. Maybe the Vitek MS RUO mode can provide a new way to analyze faster and more conveniently.

Microbiology

PA-07

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Effect of Carbon Dioxide on Biochemical Phenotype of Capnophilic ***Proteus mirabilis*** At a Regional Hospital in Southern Taiwan

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Purpose

A rare strain of carbon dioxide–dependent ***Proteus mirabilis*** was isolated from urine specimen at a regional hospital, the first isolate of capnophilic ***P. mirabilis*** at this hospital. The strain showed non–swarming and did not produce H_2S property. This study was conducted to study the biochemical phenotype of the isolate in different CO_2 atmosphere concentrations.

Methods

The capnophilic ***P. mirabilis*** as study strain (PM103), another patient in this hospital for urine culture result non–capnophilic ***P. mirabilis*** as a control strain (PM104), and the ***P. mirabilis*** ATCC 7002 as a reference strain. Three strains were inoculated on blood agar plate (BAP) and eosin–methylene blue agar (EMB), and the strains of biochemical test using conventional biochemical tests and API 20E. All tests were placed in ambient air, and in 2.5%, 5%, 7.5% and 10% CO_2 atmosphere, respectively to investigate the effect of the biochemical characteristics in different CO_2 conditions.

Results

The PM103 didn't grow in ambient air, but grew at CO_2 environment with non–swarming (FIG 1). In 5% and 7.5% CO_2 atmosphere grow best, but the CO_2 concentration continues to increase to 10% content the isolate grew poor. Regardless of the colonies grew on BAP and at any CO_2 atmosphere environment which had non–swarming. The PM104 and ***P. mirabilis*** ATCC 7002 could grow on BAP and EMB in any conditions with swarming. The results of the PM103 of conventional biochemical tests and API 20E showed no difference and without H2S property, wherever in 2.5%, 5%, 7.5% and 10% CO_2 conditions (FIG 2). The PM104 and ***P. mirabilis*** ATCC 7002 showed same results of conventional biochemical tests and API 20E with H_2S property, wherever in 2.5%, 5%, 7.5% and 10% CO_2 conditions (FIG 3).



FIG 1. PM103 grew on BAP with non–swarming characteristic.

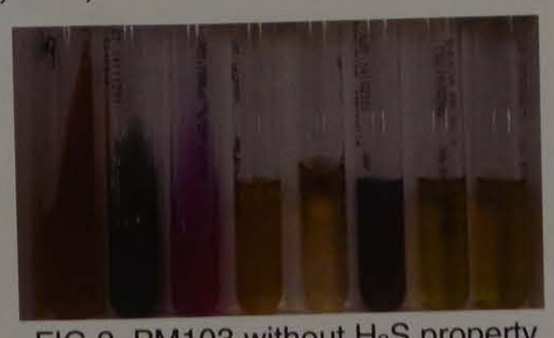


FIG 2. PM103 without H_2S property in TSI agar.



FIG 3. A:PM103 without HS property in API 20E. B:ATCC 7002 revealed H2S property in API 20E.

Conclusio

A rare capnophilic ***P. mirabilis*** strain in different CO_2 atmosphere does not change the characteristics which non–swarming and doesn't produce H_2S.

Microbiology

PA-08

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Case report-Diphyllobothriasis Parasites infection of dietary

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Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation , New Taipei City, Taiwan
Center for Diagnostics and Vaccine Development, Centers for Disease Control, Ministry of Health and Welfare, Taiwan

Objective

To know a rare case of *Diphyllobothriasis*.

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Introduction

Along with the advancement and improvement of medical and health habits, parasites infection is unusual in Taiwan. With the food culture, parasitic infections are still inevitable.
Diphyllobothrium latum is known as tapeworm, and it is the biggest parasite which can infect human.
Diphyllobothrium latum is classified to *Pseudophyllidea* of *Cestoda*. The common features of *Pseudophyllidea* are central joint genital pore, curly uterus, and having genital pore which ovulate. In addition, the features of eggs are more rounded appearance, having double shell, terminal knob, operculum and full with yolk. [1]
The native places of *Diphyllobothrium latum* are Scandinavia, Baltics, western Russia and North America. [2] However, the most cases happen in Asia, Europe, and North America. [3] The intermediate host of *Diphyllobothrium latum* is fish, such as salmon and the final host is mammals, for example human or bear. As eating raw or uncooked food, human will infect *Diphyllobothrium latum*. The eggs will appear in feces after 5-6 weeks. [4]
The symptoms are diarrhea, stomach ache, weight lossand so on. [5] The few cases will cause vitamin B12 deficiency and pernicious anemia. [6]
The way to diagnosis is to exam whether eggs are in the feces.

Results

In 2016, a 32-year-old female patient cannot discharge parasite through stool solution, she had to manually put out the parasite. There are three parasites, the longest parasite is 43 cm, and total weight is 3 gram of the three parasites. (Figure.1) Proglottids are broader than long and the black place is genital pore. (Figure.2) We suggested this is adult proglottids since the genital pore. Adding normal saline into the genital pore, we found that the genital pore above the midline of the proglottids, in according to parasites patterns determine that the pseudophyllidea preliminary. (Figure.3) Directly observing feces, we found ovoid eggs have double shell, terminal knob, and operculum. (Figure.4) The size of egg is about 60μm in length and 50μm in width. We used MIF stain to observe the eggs from the patient's feces, and the observation shows, the eggs have double shell and terminal knob. However the existence of operculum is not clear. (Figure. 5) We used three staining methods including Papanicolaou stain (Figure. 6), Trichrome stain (Figure.7) and Merthiolate-Iodine-Formalin stain (Figure. 8) to stain the proglottids. In Papanicolaou stain, we found the uterus filled with eggs, genital pore, testis and eggs. The genital pore is at the midline of the proglottids. (Figure.6) In the Trichome stain (Figure.7) and MIF stain (Figure.8), we can find some same features, which are cruel and brown uterus and the central genital pore. However, they still have different patterns, like the color of proglottids and spot-liked testis. The proglottids with Trichome stain are red and with MIF stain is brown. Without stain, we still observe cruel uterus which filled with eggs, central genital pore, and spot-liked testis. (Figure.9) Stretching the proglottids with MIF stain (Figure.10) and Trichome stain (Figure.11), we found eggs are filled with uterus. According to these features, we determined that the parasites are *Diphyllobrothrium latum*.
We asked the patient's dietary habits, and found out that she likes to eat beef and Japanese cuisine; therefore we suggested she change the dietary habits from raw food to cooked-food based, in order to avoid parasite infection in the future.

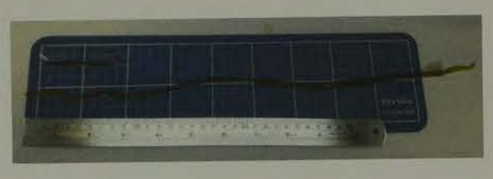


Figure.1
To know how long are the parasites



Figure.2
Proglottids are broader than long and the black place is genital pore. (This is provided by Shih-Fen Hsu.) (10x)



Figure.3
The genital pore is at the midline of the proglottids. (This technique is provided by Shih-Fen Hsu.)

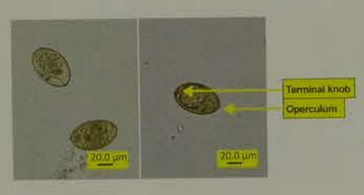


Figure.4
The egg is without stain.
(This is provided by Shih-Fen Hsu.) (400x)

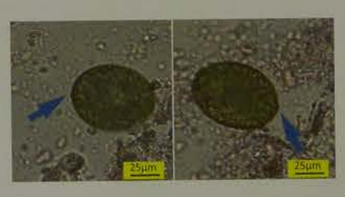


Figure.5
The eggs are stained by MIF solution. The blue arrows point at the terminal knob. (400x)

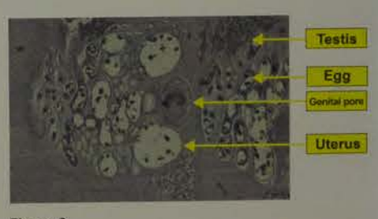


Figure.6
The proglottids are stained by Papanicolaou stain. (100x)

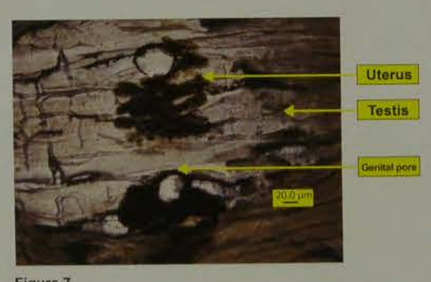


Figure.7
The proglottids are stained by Trichrome stain. (This is provided by Shih-Fen Hsu.) (40x)



Figure.8
The proglottids are stained by MIF stain.(This is provided by Shih-Fen Hsu.) (40x)

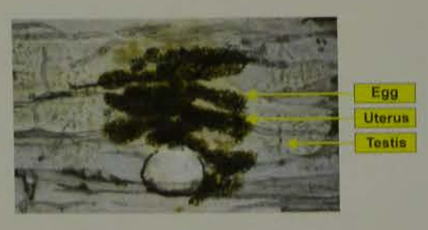


Figure.9
The proglottids are stained by MIF stain.
(This is provided by Hsiu-Maan Kuo.) (100x)
Thank you for China Medical University Departmentof Parasitology Associate Professor Hsiu-Maan Kuo's advisor and assistance.



Figure.10
Stretching the proglottids are stained by MIF stain.
(This is provided by Shih-Fen Hsu.) (100x)



Figure.11
Stretching the proglottids are stained byTrichome stain. (This is provided by Shih-Fen Hsu.) (100x)

Patients & methods

1 Patient
A 32-year-old female who likes to eat beef and Japanese cuisinehad a problem for uterine fibroids and treated for some time. She had constipation, abdominal distention and stomach ache sometimes, and her weight reduction of four kilograms in a year. For one day, she discharged parasites and took it to the hospital. We checked her laboratory data which CRP and Eosinophil did not raise with parasite infection.

2. Methods
2.1 Merthiolate-Iodine-Formalin stain
2.1.1 To observe eggs
2.1.1.1 Mix MF Stock Solution (Shih-Yung Medical Instruments Co., Ltd) with Lugol solution (Shih-Yung Medical Instruments Co., Ltd) to be a working solution.
2.1.1.2 Add stool into working solution and stain overnight.
2.1.1.3 Filter the sample into a new tube.
2.1.1.4 Add ehylaetate and intensively shake the sample.
2.1.1.5 Centrifugal the sample and remove the supernatant.
2.1.2 To observe proglottids
2.1.2.1 Add MIF solution into genital pore.
(This technique is provided by Shih-Fen Hsu.)
2.2 Papanicolaou stain
2.2.1 Wrap the parasite to a waxy section
2.2.2 Stain it by Papanicolaou stain (MUTO PURE CHEMICAL CO., LTD), 100% alcohol (Leica Mcrosysten (SEA) Pte Ltd), and 95%alcohol (SENG FA CHEMICAL BIOTECH Co., LTD).
2.3 Trichrome stain
2.3.1 Add Trichorme dye into genital pore.
(This technique is provided by Shih-Fen Hsu.)

Microbiology

PA-09

Amino Acid Determination of the Dominant Protein Isolated from Fertilized, Corticated *Ascaris lumbricoides* Egg

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Abstract

Ascariasis affects one billion people worldwide and this condition remains asymptomatic in most cases. Despite having high prevalence, it is considered as one of the Neglected Tropical Diseases (NTDs). Even with technological advancements, the most common laboratory diagnosis is still through the microscopic identification of *Ascaris lumbricoides* eggs from a stool sample. Thus, this study was conducted to determine the amino acid composition of the protein coat of fertilized, corticated *A. lumbricoides* eggs. Fertilized, corticated eggs recovered from the stool sample were concentrated and decorticated in order to separate the dominant protein from the eggs. The concentrated eggs were decorticated using the concentrations 10%, 20%, 30%, 40%, and 50% Phosphate-Buffered Saline with Tween 20. Micro-Bradford assay of the different supernatants after egg decortication revealed increasing absorbance suggestive of increasing protein content (r=0.71). Microscopic images of eggs before and after decortication were also noted. High Performance Liquid Chromatography results exhibited peaks which belong to arginine and tyrosine with concentrations of 1.627 µmol/mL and 1.678 µmol/mL, respectively. Moreover, these two amino acids are also present in proteins which are mammalian trypsin and chymotrypsin inhibitors. Information obtained from this study is significant in developing Rapid Diagnostic Tests for parasitic identification.

Keywords: *Ascaris*, Dominant Protein, Corticated

Introduction

Laboratory diagnosis of ascariasis is done through the recovery of *Ascaris lumbricoides* eggs in stool. Although detection through microscopy is relatively cheap and simple, it has a lot of limitations for it is time-consuming, labor-intensive, and has limited sensitivity and specificity (Lamberton & Jourdan, 2015). Thus, alternatives are currently studied and developed and one of these is the use of Rapid Diagnostic Tests (RDTs). The study done by Tormo and Chordi (as cited in Florkin, 2012), examined a number of purified protein-containing antigens in *Ascaris*. Several studies have proven the role of protein in *Ascaris* egg as a marker used in molecular diagnostic, serologic, and biological studies of parasitic nematodes. Although many of the studies have already shown the importance of proteins, ascariasis is still considered as a neglected tropical disease and reliably approximating the scope of the problem is difficult because of inaccuracies and imprecisions in parasitological diagnosis and the lack of definite clinical signs (Brooker & Pullan, 2013).

Objectives

The general objective of this study was to determine the amino acid composition of the dominant protein isolated from the fertilized corticated *Ascaris lumbricoides* egg.

The specific objectives are as follows:

a) To obtain a pure isolate of the fertilized, corticated *Ascaris lumbricoides* egg;
b) To decorticate and isolate the dominant protein from the recovered eggs;
c) To determine which among 10%, 20%, 30%, 40%, and 50% is the optimum concentration of PBS-Tween 20 to be used in decortication; and
d) To determine the concentration the amino acid present in the protein isolate.

Methodology

Isolation of fertilized, corticated, *A. lumbricoides* egg



The stool sample was suspended on a Brine solution with a specific gravity of 1.20. Eggs recovered using capillary catch were pooled and washed with PBS thrice to remove formalin. Aliquots containing 20 eggs each were placed in five separate Eppendorf tubes.

Decortication of Isolated Eggs



0.5 mL of 10%, 20%, 30%, 40%, and 50% PBS-Tween 20 were added on each of the Eppendorf tubes. Samples were vortex-mixed for 1 hour and 45 minutes. Sediments were then checked under the microscope. Supernatants before and after decortication were analyzed using 96-well micro-Bradford protein assay to confirm the presence of proteins on the supernatant.

Amino Acid Determination



Microwave-assisted Acid Hydrolysis

50µL of decorticated supernatant was mixed with 100 µL of 6M hydrochloric acid, placed inside an industrial microwave oven along with 100 mL of distilled water; this was done for 5 minutes set at medium high. 200 µL of distilled water was added to the hydrolyzed sample to increase volume.



FMOC– Cl Derivatization

50 µL of the sample was mixed with a 100 µL of acetonitrile. The solution was adjusted to appropriate pH by the adding 50 µL of borate buffer (40mM in water, pH 9.2), it was then derivatized using 50 µL FMOC-Cl (10mM in acetonitrile). 100 µL of this solution was injected in the system.



High Performance Liquid Chromatography

Separation was done using a C_{18} column. The mobile phase was acetonitrile and 50 mM sodium acetate solution, with a pH of 4.15. The flow rate was 1.0 mL/min and the detection wavelength used was 263 nm. The column temperature was 30°C, and the injection volume was 100 µL.

Result and Discussion



Figure 1. Microscopic Images of Fertilized, Corticated Egg of *Ascaris lumbricoides* Before and After PBS-Tween 20 Treatment

Table 1. Absorbance Difference of Supernatants Before and After Egg Decortication (r=0.71)

Supernatant Fluid	PBS-Tween 20				
	10%	20%	30%	40%	50%
Before Decortication	0.783	0.790	0.860	0.794	0.813
After Decortication	0.821	0.857	0.912	0.843	1.903
Absorbance Change	0.038	0.070	0.052	0.049	1.090

Notable changes in the morphology of the parasite eggs are observed in Figure 1. This agrees with the results obtained from the Micro-Bradford Protein Assay where the absorbance of the supernatants after decortication increased for all of the concentrations used. Moreover, among the concentrations, 50% PBS-Tween 20 was the optimum concentration since it obtained the largest increase in absorbance (Refer to Table 1). The results are parallel with the study conducted by Amirilargani, et al. (2009) where in increasing concentrations of Tween 20, there was a notable change in the membrane morphology.

Components 1 and 34 were identified as the contents of the sample. The specific identification of amino acids were done by comparing the chromatogram of the standard to the chromatogram of the standard used in the study conducted by Zhou, et al. (2011) due to the same chromatographic conditions applied. Component 1 was identified as arginine, with a concentration of 1.627 µmol/mL (Figure 2), and Component 34 was identified as tyrosine, with a concentration of 1.678 µmol/mL (Figure 3).

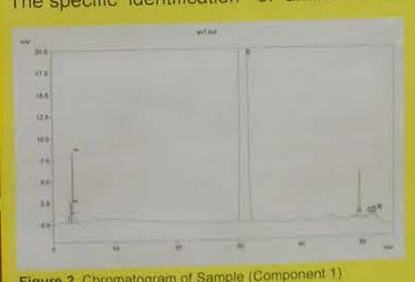


Figure 2. Chromatogram of Sample (Component 1)

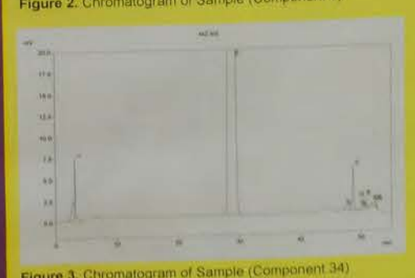


Figure 3. Chromatogram of Sample (Component 34)

As stated in Florkin (2012), there are low molecular weight proteins present in *Ascaris lumbricoides* that inhibit the activity of mammalian trypsin and chymotrypsin; in at least four of the inhibitors, tyrosine and arginine were present; the said inhibitors showed stability to heat and pH changes.

Conclusion and Recommendation

The amino acids identified and quantified as a result of the experimentation were arginine and tyrosine with the concentrations of 1.627µmol/mL and 1.678µmol/mL, respectively. These amino acids are found in low-molecular weight proteins present in *Ascaris lumbricoides* which inhibit the activity of mammalian trypsin and chymotrypsin. Decortication using 50% PBS-Tween 20 obtained the greatest change in absorbance, thus, it is the optimum concentration to be used for the isolation of the protein coat as compared to the other concentrations used in this study. Furthermore, based on the data obtained, the use of increasing concentrations of PBS-Tween 20 also increased the amount of protein recovered from the supernatant (r=0.71).

Recommendations

For further improvement of the study, the researchers recommend the following:

1. A study on the amino acid sequence
2. Development of Rapid Diagnostic Tests for the identification of *Ascaris lumbricoides*
3. Increased exposure time with 50% PBS-Tween 20 and increased amount of sample to be hydrolyzed
4. Use of concentrations higher than 50% PBS-Tween 20; and
5. Analysis of the presence of other amino acids

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Microbiology

PA-10

Determination of Infection Rate Among *Lymnaea spp.* by Cercarial Emergence in Brgy. Cawongan, Padre Garcia, Batangas

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ABSTRACT

Fasciola hepatica, commonly known as 'Sheep Liver Fluke', is the known cause of one of the global concerns in the health sector known as *Fascioliasis*[1]. Its life cycle involves two hosts: fresh water snails belonging to the *Lymnaea* spp., its intermediate host and herbivorous animals, including humans, its definitive host[1]. The study was focused in determining the natural infection rate of the *Fasciola* sp. in *Lymnaea* spp. snails through cercarial emergence. Following a completely randomized design, two rounds of collection were done for this study. Snails for both collections were obtained from Barangay Cawongan, Municipality of Padre Garcia, Province of Batangas. Perforated wide – mouthed topped containers were used as the snails' vessel. The first batch of snails (n = 147) were placed on a controlled set up consisting of adequate light and scheduled water changing. They were observed for cercarial emergence in the span of four weeks. The said batch of snails manifested a mortality rate of 20%, with an average of 31 deaths per week. . The second batch of snails (n = 431) on the other hand was divided according to their area source: behind a local pigpen and rice field. Snails measured an average weight of 0.090 g (± 0.16) and average height of 8.38 (± 3.20) mm. This batch was then immediately crushed and was subjected to three rounds of microscopic reading for the presence of *Fasciola* sp. cercariae. The first batch of snails that were observed under a controlled environmental condition for cercarial emergence showed negative results. On the other hand, the second batch of snails showed presence of free - living protozoans, operculated ova, *Ascaris* spp. egg and cyst - like structures, and sporocyst-like larval forms.

Keywords: Cercariae, *Lymnaea* spp, *Fasciola* sp.

INTRODUCTION

Fascioliasis is a known disease caused by a liver fluke and is considerably significant to public health. It has now become a global concern: in fact, according to Mas-Coma 2005, statistics showed that it has already affected 51 countries in the present.[1] It is mainly caused by the parasite *Fasciola hepatica*. However, the said parasite does not infect humans directly. Instead, it needs two hosts in order for its life cycle to be completed: the intermediate host which are freshwater snails, specifically *Lymnaea* species, and herbivorous mammals including humans as the final host. Our study focuses on its intermediate host, the snail, and the rate by which it is infected by the said parasite through cercaria which is the life stage infectious to these snails.

METHODOLOGY

I. SNAIL SAMPLING:

Field Collection



Batch 1 (n = 147)
Area 1: Rice field
Area 2: Irrigation Canal



Batch 2 (n = 431)
Area 1: Behind Local Pigpen
Area 2: Rice field

Authentication and Certification



Collected snails were authenticated and certified by the National Museum Zoology Department.

II. SNAIL MORPHOMETRICS:

Length Determination



Vernier caliper was used in measuring the live snail's external length.

Weight Determination



Shimadzu analytical balance was used in measuring the live snail's weight.

III. SNAIL PROCESSING: DETERMINATION OF INFECTION RATE

Cercarial Emmergence Setup (n=147)



- Room temperature
- Adequate lightning
- Distilled water suspension
- Scheduled water change
- Weekly recording of mortality

Crushing Method (n=429)



- Suspension of snails in tap water by using glass test tubes
- Crushing of snails with glass rods
- Tease snail's flesh and removal of debris

Microscopic Examination



- Mounting of specimens on slides
- Spread the specimens evenly
- View under LPO

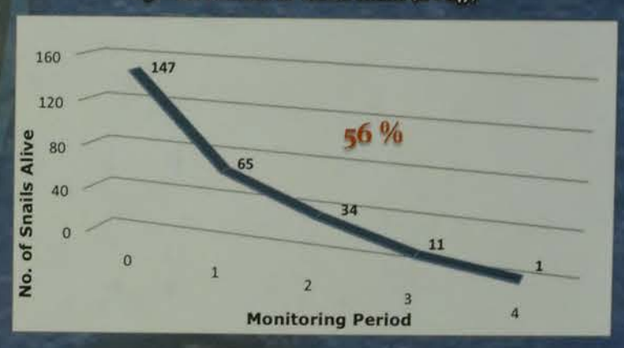
RESULTS AND DISCUSSION

Table 1. . Summary of average morphometric details of Batch 2 Snails (n = 431)

Location	Mean
Area 1: Local pigpen	
Weight	0.080 g (± 0.09)
Length	8.50 mm (± 1.61)
Area 2: Local rice field	
Weight	0.05 g (±0.02)
Length	7.14 mm (± 0.090)
Total: Area 1 and Area 2	
Weight	0.090 g (±0.160)
Length	8.38 mm (±3.20)

Table 1 shows the summary of the morphometric details of the snails collected from Area 1 (local pigpen) and Area 2 (local rice field) of Batch 2. For Area 1: the average weight is 0.08 g (± 0.09) and its average length is 8.50 mm (± 1.61). On the other hand for Area 2, the average weight is 0.05 g (± 0.02) and its average length is 7.14 mm (± 0.090). All collected snails had an average weight of 0.090 g (± 0.160) and an average length of 8.38 mm (± 3.20).

Figure 1. Number of Viable Snails (n = 147)



As seen in Figure 1, A sharp 56% mortality was observed after 1 week storage of collected snails under laboratory setting for cercarial emergence and a downward trend continued over time.

Figure 2. Preliminary Identification of Microscopic Findings (x100)

Free - living Protozoans



Helminthic Eggs



Precercarial Forms



Figure 2 shows microscopic images of organisms initially identified based on their general characteristics. They are clustered as free-living protozoans, helminthic eggs, cyst-like and sporocyst-like larval forms.

CONCLUSION AND RECOMMENDATION

There was no cercariae observed in the whole batch of snails (n=576) collected. However, presence of free-living protozoans, nematodes in different life cycle stages, cyst - like structures and operculated ova, which are suspected to be of *Fasciola* sp. were noted on the second batch of snails (n=431) that were sampled and crushed.

The group recommends:

- Environmental temperature as a major contributing factor to the cercarial emergence
- Molecular confirmation of detected parasites
- Induce cercarial emergence through various stimulants

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Microbiology

PA-11

Development of *Ascaris lumbricoides* in the Gut of Local Cockcroaches (*Periplaneta americana*)

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[1]University of Santo Tomas, Faculty of Pharmacy , Department of Medical Technology

ABSTRACT

Currently, intestinal helminthiasis affects an estimated 500 million to 1 billion people yearly, at least 400 million children of school age[1]. Recent studies show that the cockroaches are actual carriers of medically relevant parasites causing serious health problems[2]. They act as mechanical vectors as they carry parasites in their external and internal parts, disseminating them in the environment from ingestion of contaminated human feces. Eighty-four cockroaches were collected for the study. They were placed on an artificial environment containing bread with stool positive for *Ascaris lumbricoides*. Any gross morphological changes that occur to them were monitored in a four week interval after which the gut was removed, dissected, and examined for the presence of various developmental changes of the parasite. Based on the results, there was an increased mortality rate observed in the experimental group over a three week monitoring period from week 1 (42.86%) up to week 3 (76.19%). All infected cockroaches showed a marked increase in their weight. Post-euthanized infected cockroaches revealed the presence of motile *Ascaris* larva suggestive of continued viability of *Ascaris lumbricoides*. In conclusion, local cockroaches were capable of supporting the life of *Ascaris lumbricoides*. There was a positive correlation between the weight of the cockroach and the severity of the infection. Mortality rate of the cockroaches increased possibly due to the lack of adequate resources inside an isolated environment and succumb to infection. In the light of these findings the researchers recommend the measurement of the larva developed within the gut through ocular micrometry and associate it with its life span and viability.

Keywords: *Ascaris lumbricoides*, cockcroach, light microscopy

INTRODUCTION

Cockroaches are some of the most ubiquitous insects in the world and are considered potential carriers of several medically relevant parasites causing serious public health problems . Cockroaches can act as mechanical vectors as they carry parasites in their external and internal parts disseminating them in the environment through ingestion of contaminated human feces.

Our study aims to determine whether a parasite as prevalent in the Philippines like *Ascaris lumbricoides* can grow within the internal organs of cockroaches . This includes monitoring weekly the development of the parasitic helminth within the gut of the cockroaches by counting the numbers of eggs/larva and by monitoring the changes that occurred to the cockroach before and after experiment.

METHODOLOGY



1 Preparation of Artificial Environment



2 Collection of 84 cockroaches and storage in containers



3 Euthanasia by insecticide



4 Measurement of cockroach morphometrics



5 Dissection of euthanized cockroaches



6 Maceration of the gut and centrifugation



7 Microscopy of emerged larva and eggs

RESULTS AND DISCUSSION

Figure 1. Mortality of the Four Groups

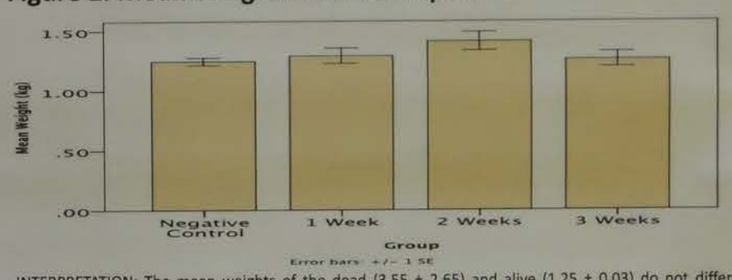


Mortality	*Group* Negative Control	One Week	Two Weeks	Three Weeks	p-value	*pairwise comparison*
Dead	3 (14.3%)	9 (42.9%)	10 (47.6%)	16 (76.2%)	<0.001	Neg<(W1=W2=W3)
Alive	18 (85.7%)	12 (57.1%)	11 (52.4%)	5 (23.8%)		

Table 1. Mortality of the Four Groups

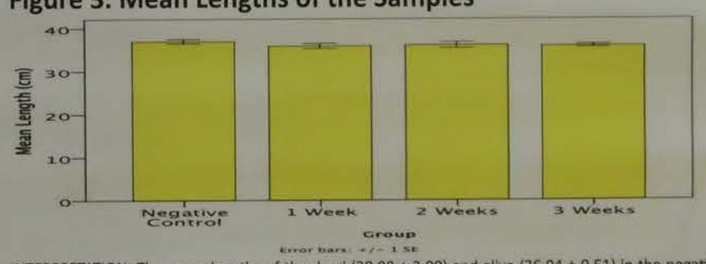
INTERPRETATION: The mortality rate significantly differ (p<0.001) according to group. This indicates that the mortality rate of the negative control group (14.3%) is significantly less (p=0.043) with one week group (47.6%). The mortality of negative control group is also significantly less than the (p=0.022) with two weeks group (47.6%) and three weeks group (p<0.001; 76.2%). Moreover, the mortality rates of the one week, two week, and three week group do not differ (p=1.000).

Figure 2. Mean Weights of the Samples



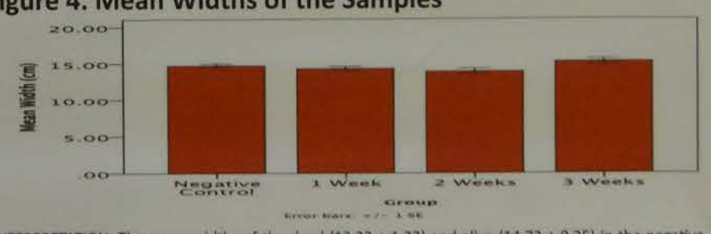
INTERPRETATION: The mean weights of the dead (3.55 ± 2.65) and alive (1.25 ± 0.03) do not differ (t_2=0.867, p=0.477) in the negative control group. Similarly, the mean weights of the alive samples in the negative control group (1.25 ± 0.03), one-week group (1.29 ± 0.06), two-week group (1.41 ± 0.08) and three-week group (1.26 ± 0.06) do not differ [$F_{3,42}$=1.750, p=0.172].

Figure 3. Mean Lengths of the Samples



INTERPRETATION: The mean lengths of the dead (38.00 ± 2.00) and alive (36.94 ± 0.51) in the negative control do not differ (t_{19} = 0.727, p=0.476). Likewise, the mean lengths of the alive samples in the negative control group (36.94 ± 0.51), one-week group (35.83 ± 0.58), two-week group (36.00 ± 0.71) and three-week group (35.80 ± 0.37) do not differ [$F_{3,42}$=0.951, p=0.425].

Figure 4. Mean Widths of the Samples



INTERPRETATION: The mean widths of the dead (13.33 ± 1.33) and alive (14.72 ± 0.25) in the negative control do not differ ($t_{2.1}$=1.023, p=0.407). Moreover, the mean widths of the alive samples in the negative control group (14.72 ± 0.25), one-week group (14.33 ± 0.28), two-week group (13.91 ± 0.37) and three-week group (15.20 ± 0.37) do not differ [$F_{3,42}$=2.186, p=0.104].

FINDINGS	*Groups* Negative Control	One Week	Two Weeks	Three Weeks	*p-value*
Number of Alive	18	12	11	5	
With Larva/Egg Findings	3 (16.7%)	12 (100%)	11 (100%)	5 (100%)	<0.001
with Larva only	3 (16.7%)	11 (91.7%)	11 (100%)	5 (100%)	<0.001
	-	4.08 ± 1.03	11.27 ± 4.03	6.20 ± 1.24	0.182
with Eggs only	1 (5.6%)	8 (66.7%)	1 (9.1%)	0 (0%)	0.001
	-	2.42 ± 0.97	0.09 ± 0.09	0	0.037
Unidentified Organism	2 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0.600
No Findings	15	0	0	0	-

Table 2. Larvae and Eggs Finding in the Four Groups

INTERPRETATION: The percentage of samples with larva/egg findings significantly differ (p<0.001), indicating that the one-week group (p<0.001), two-week (p<0.001) and three-week group (p=0.001) are all significantly higher than the control group, while the one-week, two-week, and three-week group do not differ (p=1.000). In addition, the mean number of larvae found in the one-week group [4.08 ± 1.03], two-week group [11.27 ± 4.03], and three-week [6.20 ± 1.24] group does not significantly differ [$F_{3,9.2}$=1.944, p=0.182].

CONCLUSION

- The gut of the cockroach is capable of supporting the life of a parasite. It was seen microscopically that eggs and larva appear within them after a week of infection.
- There was no significant difference between the weight of the cockroaches and the severity of the infection.
- The severity of the infection varied among all the cockroaches as the time of infection increases.
- There was an increase in mortality rate of the cockroaches. This increase can be correlated with the length of the infection period.

RECOMMENDATION

- Laboratory infection of *Periplaneta americana* with other parasites: protozoans and other STH
- Ocular micrometry to monitor developmental changes of the parasite within the host
- Use of other local species of cockroaches as vectors of parasites

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Microbiology

PA-12

In vitro Ovicidal Activity of Lansium domesticum (Meliaceae) Bark Extracts on Ascaris lumbricoides

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ABSTRACT

Intestinal worm infestations, primarily caused by *Ascaris lumbricoides*, have become one of the causes of malnutrition among Filipino children. These soil-transmitted helminths are developing resistance against commonly used anthelminthic drug treatments due to an increase in the dosing and repeated use of the same drug. Subsequently, re-infection against these helminths have also developed over time. These alarming situations demand a search for alternative treatments. Studies have shown that the bark of *Lansium domesticum* was effective in treating pain in the gastrointestinal tract caused by intestinal worm infestations. This study evaluated the ovicidal effect of *Lansium domesticum* through in vitro testing against *A. lumbricoides*. The methanolic bark extract of *L. domesticum* was obtained through percolation and the crude extract was fractionated using chloroform, ethyl acetate, and n-butanol. The extracts and the fractions were evaluated through phytochemical testing and quantification of condensed tannin content (CTC) using gallic acid as standard. The results showed that crude extract and ethyl acetate fraction yielded the highest condensed tannin content while the chloroform and n-butanol (p=0.289) yields to almost the same condensed tannin content. The CTC of the crude extract, ethyl acetate, chloroform and n-butanol fraction were 1.110, 0.871, 0.557 and 0.434 mg GAE/g sample, respectively. The Egg Hatch Test was used to assess the effect of each crude extract and fractions on the viability of *A. lumbricoides* eggs. After 48 hours, crude extract (120 mg/mL) was most effective exerting ovicidal activity up to 96 hours similar to the standard Albendazole (60 mg/mL) drug (p=0.104). Other extractives like Ethyl acetate (60mg/mL) and Chloroform (120mg/mL) showed delayed but apparent results against the ova (p=0.470). However, all the rest of the extracts did not show positive effect after incubation. The Ova Analysis was used to assess the effect of the extractives on larvae development after incubation in soil setup. Albendazole (60mg/mL) was the most effective test solution among the groups (p=0.124) followed by the crude (120mg/mL), ethyl acetate 60 (mg/ml) and chloroform 120 (mg/mL) which showed similar effect against the development of eggs (p=0.052). All the other extractives and the normal saline solution did not exhibit the desired effect (p=0.321). In vitro studies reveal that crude, ethyl acetate and chloroform (120 mg/mL) of *L. domesticum* exhibited ovicidal effect against *A. lumbricoides* and may therefore be explored as a potential alternative treatment to commercially available anthelmintic agents.

INTRODUCTION

Intestinal worm infestation is one of the most common causes of malnutrition in Filipino children. In the study by Belizario, et al (2010), *Ascaris lumbricoides* is the primary cause of the disease. The most frequently used anthelmintics to control soil transmitted helminth (STH) infections as recommended by the World Health Organization are benzimidazoles such as mebendazole and albendazole. However, resistance alleles have been found in human STH leading to reduced efficacy of anthelmintic drugs and subsequent re-infection with the parasites after treatment with benzimidazoles (Vercruysseet, et al, 2011; Saathoff, et al, 2004). This also occurs after treatment with triple-dose albendazole (Yap, et al, 2013). Due to these disadvantages of benzimidazoles, alternative treatment must be sought. Numerous extracts of different plant parts are being utilized against helminths that cause human infestations. Their anthelmintic activity is attributed to the presence of condensed tannins against *Ascaris* (Fakae, et al, 2000; Nalule, et al, 2013). *Lansium domesticum* is widely available in the Philippines. The powdered seeds of this plant were traditionally given to children against worms. However, bitter substances and traces of an alkaloid were isolated from the seeds, rendering them toxic. On the other hand, the bark which contains much tannin was used to treat colic or pain in the gastrointestinal tract that is mostly caused by intestinal worm infestation (Hajong, 2013). This study aims to determine the ovicidal effect of bark extract of *TT* as potential alternative treatment against *Ascaris lumbricoides* egg.

METHODOLOGY

Preparation of Test Solution

Percolation using 80% methanol and Fractionation using chloroform, ethyl acetate and n-butanol of Bark Extract

- Methanolic Crude Extract
- Chloroform Fraction
- Ethyl Acetate Fraction
- n-Butanol Fraction

→ Phytochemical Screening of the presence of Tannins, Saponins, Flavonoids, Alkaloids and Anthraquinones

→ Quantification of Tannins using the Modified Vanillin Assay

Preparation of Egg Suspension

Stool Sample from infected host → Direct Fecal Smear Positive stool → Egg mixture → Modified Concentration Technique → Egg Suspension → Capillary Catch

Ova Analysis Procedure

Inoculate the fertilized *A. lumbricoides* eggs on the test solutions

- Albendazole (Positive Control)
- Normal Saline (Negative Control)
- Methanolic Crude Extract
- Chlorofrom Extract
- Ethyl acetate Extract
- n-Butanol Extract

→ Homogenize mixture using vortex mixer

- Egg Hatch Assay → Conical Tube Set-up (Triplicate) → Incubation at room temperature → Observe eggs under the microscope after 48 and 96 hours
- Soil Inoculation Assay → Soil Set-up (Triplicate) → Incubation at room temperature → Direct Soil Smear under the microscope after 2 weeks

RESULTS AND DISCUSSION

Phytochemical Screening of Bark extracts and Fractions

Metabolite	Crude	Fractions		
		Chloroform	Ethyl acetate	n-Butanol
Tannins (CT)	+	+	+	+
Saponins	+	+	+	-
Flavonoids	+	+	+	-
Alkaloids	+	-	-	+
Anthraquinones	+	-	-	-

(+): Presence of the metabolite in the extract/fraction
(-): Absence of the metabolite in the extract/fraction
(CT): Presence of Condensed Tannin based on the color intensity shown in the tannin test

Condensed Tannin Content of Different Extractives

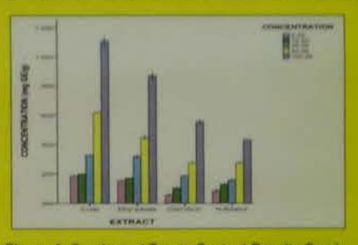


Figure 1. Condensed Tannin Content Results Graph

The different concentrations of the crude and ethyl acetate gave the highest condensed tannin content which was found to differ significantly (p<0.05) from the other groups, while chloroform and n-butanol (p=0.289) shows no significant difference yielding almost the same condensed tannin content.

The following effects were observed on the eggs after 48 hours **(Figure 2a)**, after 96 hours **(Figure 2b)**, and after 2 weeks of exposure **(Figure 2c)** on the most effective crude extract 120mg/ml.



Figure 2a. Intact egg shell **2b.**Egg distorted **2c.**Larva developed

Egg Hatch Assay

After 48 Hours

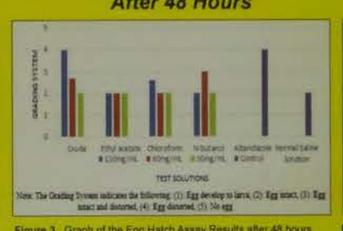


Figure 3. Graph of the Egg Hatch Assay Results after 48 hours

After 48 hours, results show significant difference which reflected variability in the effects of the fractions on the *Ascaris lumbricoides* eggs (x2= 29.244, p= 0.006) as illustrated in Figure 7. Albendazole 60mg/mL and Crude 120mg/mL shown no significant difference in the activity which resulted to the highest ovicidal effect (p=0.104) against the development of eggs. All the other extractives and normal saline solution showed no significant difference in the activity (p=0.613) against *Ascaris lumbricoides* egg.

After 96 Hours



Figure 4. Graph of the Egg Hatch Assay Results after 96 hours

After 96 hours, the different test solutions exhibited significant difference (x2= 35.345, p= 0.001) which resulted to variable effect against egg development as illustrated in Figure 8. Albendazole 60mg/mL, Crude 120mg/mL, Ethyl acetate 60mg/mL and Chloroform 120mg/mL did not shown any significant difference in its effect which exhibit same ovicidal activity (p=0.470) against the development of eggs. All the other extractives and normal saline solution yield no significant difference in the ovicidal activity against egg development (p=0.143).

Soil Inoculation Assay

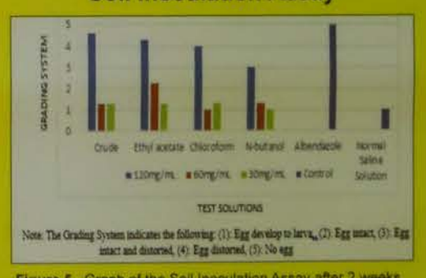


Figure 5. Graph of the Soil Inoculation Assay after 2 weeks

After two weeks of incubation period, the different test solution yielded significant difference (x2=29.982, p = 0.005) in the effects of the different test solution against the development of egg to larva as illustrated in Figure 9. Albendazole 60mg/mL is the most effective test solution among the groups (p=0.124) followed by the crude 120mg/mL, ethyl acetate 60mg/mL and chloroform 120mg/mL which shows no significant difference in the ovicidal effect against the development of eggs (p=0.052). All the other extractives and the normal saline solution with no significant difference did not exhibit the desired effect (p=0.321).

CONCLUSIONS

The *Lansium domesticum* bark was extracted with methanol and the crude extract obtained was fractionated with three solvent systems: chloroform, ethyl acetate and n-butanol. The bark extract and fractions were tested for the presence of tannins, saponins, flavonoids, alkaloids and anthraquinones. The crude extract was positive for all the constituents while for the chloroform, ethyl acetate, and n-butanol fractions, the phytochemical constituents were variable. The condensed tannin content of the bark extract and fractions were quantified using modified vanillin assay with gallic acid as standard. The crude extract at the concentration of 120mg/ml was found to have the highest content of condensed tannins. Among the fractions, ethyl acetate yielded the highest amount of condensed tannins, as compared to chloroform and n-butanol. From the egg hatch assay, it can be concluded that the positive control, albendazole, is effective after 48 hours of observation and its effect persists until after 96 hours. The effect of the crude extract at 120mg/ml was comparable to the effect of albendazole after 48 hours of observation. All of the other extractives exhibited significant effects against the eggs only after 96 hours of observation. In the ova analysis, the crude extracts along with the ethyl acetate fractions have shown to possess ovicidal activity parallel to the effect of albendazole. In both tests that were conducted, the extracts that have the most potent activity against the ovum of *A. lumbricoides* were those extract which have the highest yield of condensed tannins, and the extracts which exhibited the least activity have a low yield of condensed tannins. Therefore, confirming the study conducted by Williams (2014) in relation to the ovicidal activity.

RECOMMENDATIONS

For further studies, the recommendation will be to isolate the condensed tannin content from different extractives, validate the effect of the extracts on adult worms, and test if the extracts are also effective on other helminths. Testing in higher concentrations is also recommended as it will help determine the optimal dose for the ovicidal activity of the extract and its toxicity. In vivo studies are also advised to test the effect of the extractive on the body system.

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Microbiology

PA-13

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Gram Staining Offers Simple and Real-Time Diagnosis of *Malassezia* folliculitis: A Single-Center Series

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Background

Malassezia folliculitis is a common skin disease caused by the hyperproliferation of the fungus, *Malassezia* spp. in the hair follicle. It is usually diagnosed on clinical grounds, but its distinction from common mimickers, such as acne vulgaris and bacterial folliculitis, is crucial. Gram staining visualizes both bacteria and the blastospores of *Malassezia spp.;* therefore, it has the potential to resolve diagnostic difficulties. However, it is only rarely used in the past for such purpose. Its accuracy in diagnosing *Malassezia* folliculitis is also unknown based on past literature.

Patients and methods

To determine the diagnostic accuracy of gram staining in *Malassezia* folliculitis, we retrospectively included 32 patients, between June 2015 and January 2016, in whom gram staining was performed to diagnose *Malassezia* folliculitis. We quantified the amount of *Malassezia* blastospores on gram-stained slides and compared the results with final diagnosis, which was determined by skin biopsy (if available) and positive treatment response (if a skin biopsy was not done). We also calculated the sensitivity and specificity of both gram staining and skin biopsy.

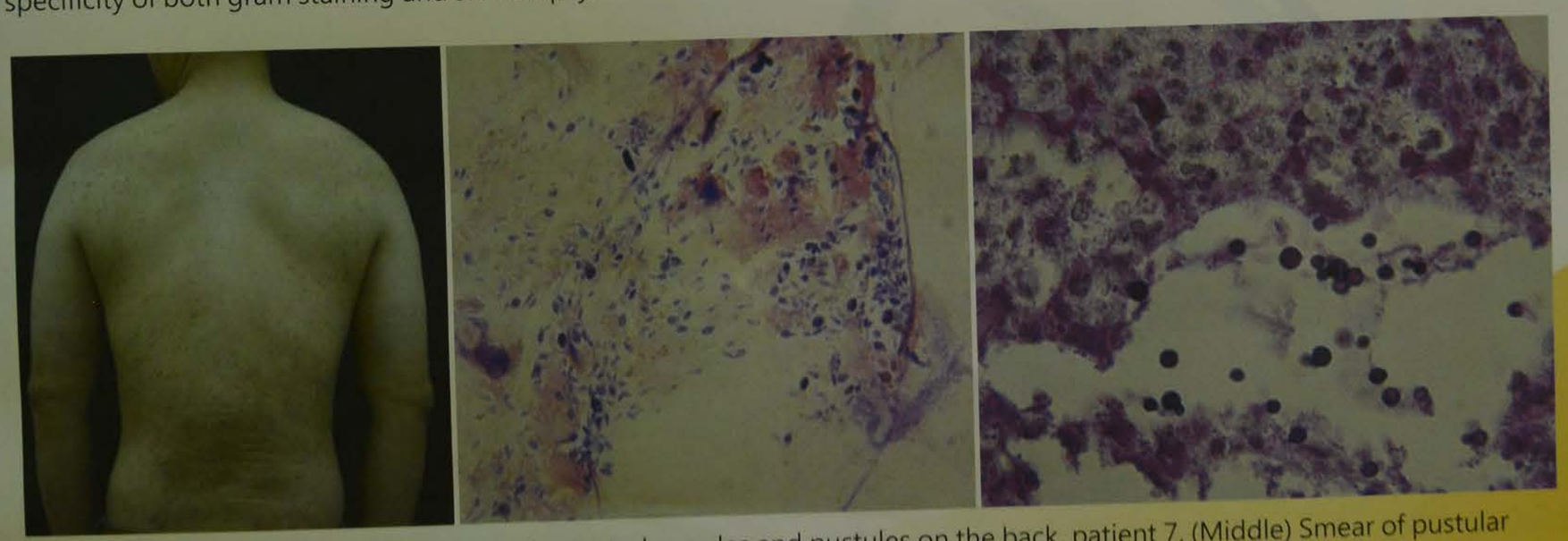
Results

In the 32 patients, 26 (81.3%) were diagnosed with *Malassezia* folliculitis, 2 (6.3%) were diagnosed with bacterial folliculitis, and 4 (12.5%) were diagnosed with mixed bacterial and *Malassezia* folliculitis. In 5 patients, gram staining results were different from the final diagnosis. The sensitivity and specificity of gram staining in the diagnosis of *Malassezia* folliculitis were 84.6% and 100%, respectively, compared to the 75% sensitivity and 100% specificity of skin biopsy.

Conclusions

In this study, we showed that Gram staining has good sensitivity and excellent specificity in diagnosing MF, which is similar to those of skin biopsy.

Based on our results, gram staining seems an easy and under-utilized method to diagnose *Malassezia* folliculitis. The presence of *Malassezia* blastospores but not bacteria is highly suggestive of *Malassezia* folliculitis. We encourage large-scale studies and comparison studies to consolidate our findings.



(Left) Numerous erythematous to hyperpigmented papules and pustules on the back, patient 7. (Middle) Smear of pustular content shows numerous variably staining, budding yeasts and hyphae, morphologically consistent with *Malassezia* spp. (Gram stain, 1000X magnification) (Right) Histopathologically, there are multiple round or unipolar, budding *Malassezia* blastospores within the inflammed hair follicle (PAS, 1000X magnification)

Microbiology

PA-14

Comparison of the Accuracy of Vitek MS and Conventional Vitek 2 System with DNA Sequencing Analysis in the Identification of Clinical Candida Isolates Collected from Sterile Body Sites

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Background

Matrix-assisted laser desorption ionization-time of flight mass spectrometry(MALDI-TOF MS) system may become a fast and reliable tool for rapid fungal identification in routine clinical laboratories. The aim of this study was to evaluate the efficacy of Vitek MS in the identification of clinical isolates of Candida species cultured from blood or sterile body sites from the patients in a major teaching hospital of northern Taiwan.

Material / methods

Fifty-nine clinical isolates were analyzed by Vitek-2, Vitek MS and internal transcriber spacer 1 (ITS1) rDNA sequencing. The efficacy of the Vitek MS was determined by comparison with Vitek 2 and ITS1 rDNA sequencing analyses.

Results

According to the results of ITS1 rDNA sequencing analyses, the 59 isolates were the followings: 11 isolates of *C. albicans*, 10 *C. tropicalis*, 12 *C. parapsilosis*, 10 *C. glabrata*, 4 *C. krusei*, 5 *C. guilliermondii*, 2 *C. lusitaniae*, 2 *C. intermedia*, 1 *C. rugosa*, 1 *C. pararugosa and* 1 *C. haemulonii* (Table1&2). By using the gene sequencing as the reference method, the correct species identification rates for Vitek MS and Vitek 2 were both 91.5% (54 / 59) (Table2). The only isolate of *C. pararugosa* was misidentified by Vitek 2 and unidentified by Vitek MS system. The other 4 Vitek 2 misidentified isolates were correctly identified by Vitek MS, and the other 4 Vitek MS unidentified isolates were correctly identified by Vitek 2.

Figure 1. Work flow of Vitek MS



Candida spp.	Total No. of isolates (%)	No. of study isolates (%)
C. albicans	161 (56.9)	10 (16.9)
C. parapsilosis	40 (14.1)	10 (16.9)
C. tropicalis	34 (12.0)	10 (16.9)
C. glabrata	29 (10.2)	10 (16.9)
C. krusei	4 (1.4)	4 (6.8)
C. famata	4 (1.4)	4 (6.8)
C. guilliermondii	4 (1.4)	4 (6.8)
C. lusitaniae	2 (0.7)	2 (3.4)
C. intermedia	2 (0.7)	2 (3.4)
C. rugosa	2 (0.7)	2 (3.4)
C. haemulonii	1 (0.4)	1 (1.7)
Total	283 (100)	59 (100)

Table 1. Species distribution of Candida isolates obtained from blood or sterile body sites in 2009, identified by Vitek-2 system

Candida spp.	No. of isolates by ITS 1	No. (%) of isolates with consistent results*	
		Vitek MS	Vitek 2
C. albicans	11	11 (100)	10 (91)
C. tropicalis	10	9 (90)	10 (100)
C. parapsilosis	12	10 (83)	10 (83)
C. glabrata	10	10 (100)	10 (100)
C. krusei	4	4 (100)	4 (100)
C. guilliermondii	5	5 (100)	4 (80)
C. lusitaniae	2	1 (50)	2 (100)
C. intermedia	2	2 (100)	2 (100)
C. rugosa	1	1 (100)	1 (100)
C. pararugosa	1	0 (0)	0 (0)
C. haemulonii	1	1 (100)	1 (100)
Total	59	54 (91.5)	54 (91.5)

Table 2. Comparison of Vitek MS and Vitek 2 system. * compared with ITS 1.

Conclusions

In conclusion, the Vitek MS system is comparable to Vitek 2 in the identification of clinically important isolates of Candida species.

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Microbiology

PA-15

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Comparison of Routine Microbiological Methods for Identification of Candida Species

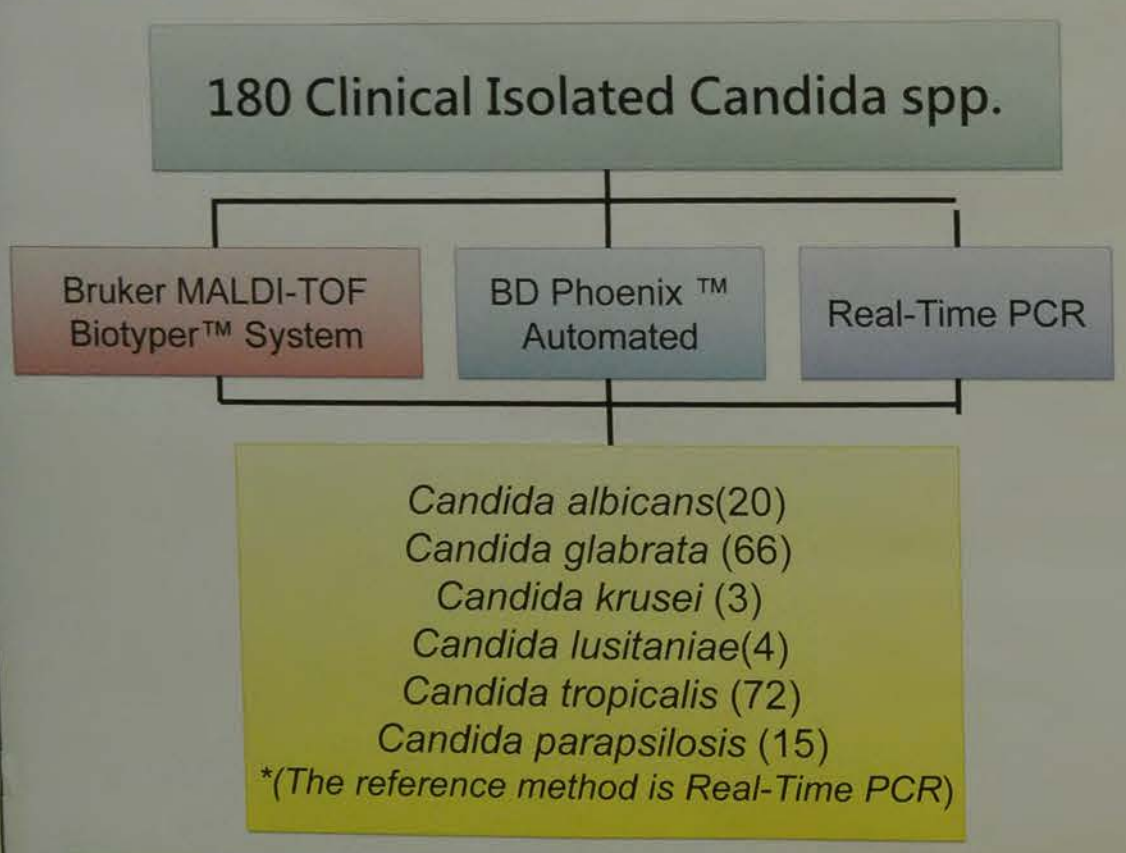
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Introduction

Candida albicans and other Candida spp. are the most common fungal pathogens causing fungal infections. Opportunistic fungal infections are a major cause of morbidity and mortality in immunocompromised patients such as transplant recipients. The clinical features of invasive candidiasis are nonspecific, making early diagnosis of invasive candidiasis difficult. Recently, the incidence of healthcare associated bloodstream infections caused by Candida spp. has increased in immunocompromised patients. Non-cultural techniques used previously have lacked sensitivity and specificity. New methods has been applied as a rapid, accurate and inexpensive method for the detection of fungal infection early in the course of disease.

Methods



Results

The consistency between the both Phoenix system and Real-time PCR for identification of *C. albicans*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. tropicalis*, *C. parasilosis*, were 89%, 94%, 100%, 100%, 89%, and 73%, respectively. The consistency between the both MALDI Biotyper™ System and Real-time PCR fo identification of *C.albicans*, *C.glabrata*, *C.krusei*, *C. lusitaniae*, *C. tropicalis*, *C.parasilosis*, were 74%, 96%, 100%, 100%, 85%, and 73%, respectively. In addition, the mass spectra of MALDI Biotyper™ System were applied for constructing dendrogram, which shows six cluster groups with a default critical distance level of 700.

	Phoenix(%)	MALDI-TOF(%)
C. albicans	89	74
C. glabrata	94	96
C. krusei	100	100
C. lusitaniae	100	100
C. tropicalis	89	85
C.parasilosis	73	73

Table 1. The consistency between the both Phoenix system and Real-time PCR for identification of six Candida spp.(Left table) ; The consistency between the both MALDI Biotyper™ System and Real-time PCR fo identification of six candida spp.(Right table)

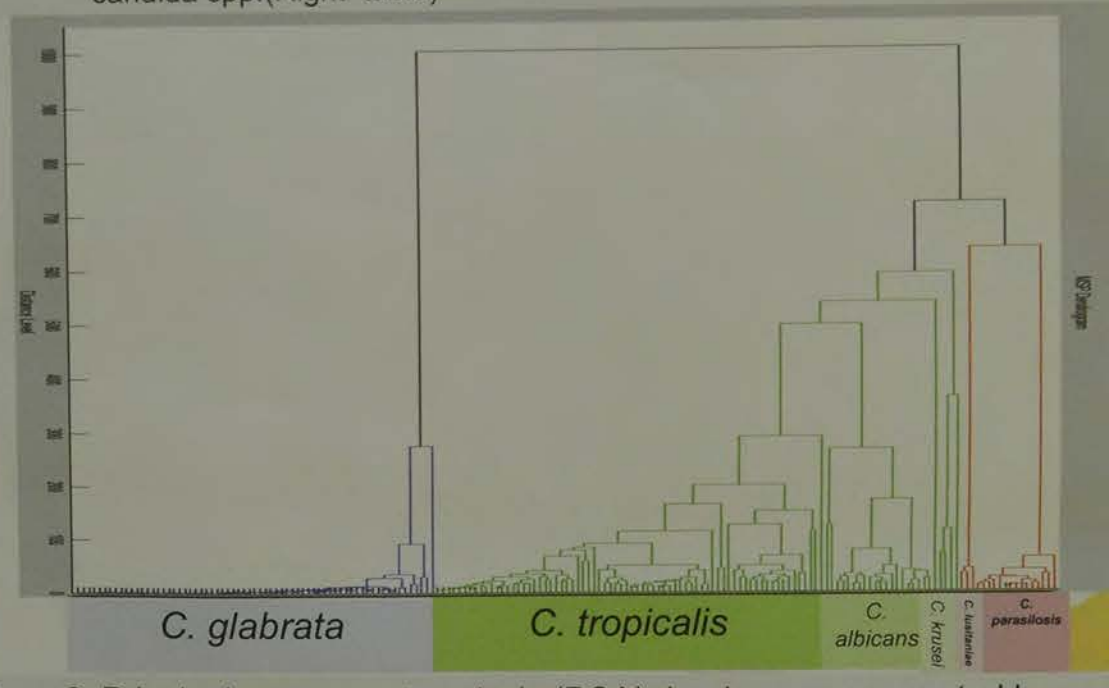


Figure2. Principal component analysis (PCA) dendrogram generated by MALDI Biotyper mass spectra for 180 isolates of six Candida species.

Conclusion

This study provides us the efficacies of Automated Microbiology System, MALDI Biotyper™ System, and the real-time PCR methods for identification of Candida spp. from clinical samples.

Microbiology

PA-16

台南市立醫院
Tainan Municipal Hospital

Laboratory investigation of suspected in–vivo acquisition of antibiotic–resistant genes In–vivo acquisition of antibiotic–resistant genes

Chao-Tai Lee
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Purpose

At a regional hospital in southern Taiwan, on 16 March 2014, one isolate of carbapenem–resistant *Providencia stuartii* (CRPS, P2) and one isolate of carbapenem–resistant *Klebsiella pneumoniae* (CRKP, K1) were isolated from the same urine sample from a patient came from nursing home. In addition, another isolate of CRPS (P1) was isolated from this patient on 14 February 2014. Because two isolates of carbapenem–resistant Enterobacteriaceae coexisted were unusual, we investigated the relationship of antibiotic–resistant mechanisms of P2 and K1.

Methods

A surveillance culture for environmental objects and healthcare workers (HCWs) in the Department of Ophthalmology were involved. The isolate was identified using the Phoenix automatic system (Becton Dickinson Diagnostic Systems, Sparks, MD). The standard disk diffusion was performed for antimicrobial susceptibility testing, and interpretive criteria according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI).

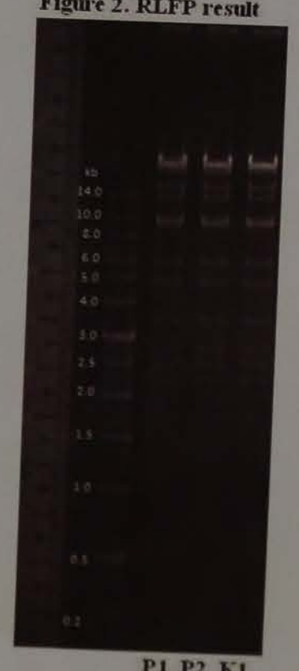
Results

PFGE pattern revealed P1 and P2 were the same strain (Figure1). A 200 kb IncA/C plasmid was present in both P2 and K1, which carried genes of bla_{CMY-2}, qnr, and *aac(6')-Ib-cr*. RFLP revealed that the 200 kb plasmids of P1, P2, and K1 were almost same (Figure2).

Figure 1. PFGE result



Figure 2. RLFP result



Conclusions

As a result of this study, the antibiotic–resistant plasmid of K2 might come from P2 by in–vivo acquisition. Although clonal spread was an important cause of high rate of antibiotic–resistant organisms in long–term care facilities, this study revealed that the spread of antibiotic–resistant genes was also an important cause.

Microbiology

PA-17

台南市立醫院
Tainan Municipal Hospital

To investigate an outbreak caused by carbapenem and tigecycline–resistant *Enterobacter cloacae*

Chao-Tai Lee
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Purpose

At a regional hospital in southern Taiwan, 11 isolates, including 8 from community and 3 from hospital, of carbapenems and tigecycline–resistant *Enterobacter cloacae* were isolated from September 2010 to April 2012. Because they were unusual, we investigate suspected an outbreak caused by *E. cloacae* by molecular methods.

Methods

Antimicrobial susceptibility testing was performed by E–test. The antibiotics tested included imipenem, meropenem, doripenem, and tigecycline. All results were interpreted according to the criteria suggested by the Clinical Laboratory Standards Institute 2015, but tigecycline was according to that by the European Committee on Antimicrobial Susceptibilities Testing 2015. Pulsed–field gel electrophoresis (PFGE) was used for bacterial genotyping. PFGE patterns were interpreted as same (no band difference), similar (≦three–band differences), or different (≧four–band differences) strains.

Results

All of the 11 isolates of *E. cloacae* were nonsusceptible to at least one of imipenem, meropenem, and doripenem as well as resistant to tigecycline. PFGE patterns revealed 5 genotypes were present (Figure 1), including 1 A (E1), 5 B (E2–E6), 3 C (E7–9), 1 D (E10), and 1 E (E11).

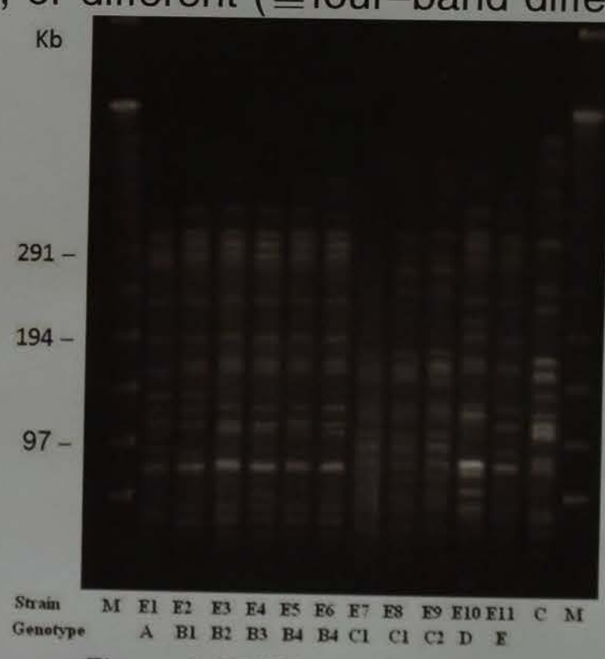


Figure 1.PFGE patterns of the 11 isolates of *E. cloacae*

Conclusions

As a result of this study, these were outbreaks caused by two *E. cloacae* clones, including genotype B and C. Herein, we suggest that investigating outbreaks by molecular methods should be done once encountering unusual increasing isolates of the same species with multidrug–resistance.

Microbiology

PA-20

PA-18

Identification of bacterial flora in pediatric UTIs: a comparison of results between VITEK2 and VITEKMS

Author: Claudia Finocchi (Biomedical and Microbiological Laboratory AOUMeyer, Firenze, Italy-ANTeL)

INTRODUCTION

Few processes present greater difficulties in the way of diagnosis than the identification of an infecting microorganism and the most effective therapy for it. The Meyer Children Hospital in Florence is currently carrying out a three-hour growth test in broth medium and analyzed in turbidimetry (ALFRED60, ALIFAX) in order to distinguish the positive samples from the negative ones: positive urines (in pediatrics the cut off is 30000 CFU/mL instead of 100000 UFC/mL) are then plated and incubated for at least 24 hours. In addition, the identification and the resulting antibiogram from VITEK2 (Biomérieux) last at least 9 to 15 hours. So, the report will be available in 48/72 hours after the arrival of the samples at the laboratory, increasing the waiting time to give the right therapy to the child.

AIM

This research has considered the possibility of making the identification straight from the culture medium for screening with the subsequent analysis of pellet from centrifuged samples and, finally, analyzed by VITEK-MS (MALDI-TOF Technology, Biomérieux). The main aim of this work is to decrease at least the time of identification of the bacteria due to dose the pediatric patients with the proper antibiotic in the shortest time from the urine culture positivity.

METHOD IN USE

Screening: ALFRED60

500µL of sterile urine are dispensed in a 2mL glass vial with enriched broth medium. The incubation of the samples at 37°C lasts 3 hours. During this time, ALFRED60 provides a "Light Scattering" reading. The laser is able to read the turbidity through the vial every five minutes and to get the evenctual bacterial growth. The signal is revealed by two detectors placed one at 30°, giving the sensitivity, and the other at 90°, indicating the specificity. This technology evaluates the growth of live bacteria and does not consider the interference of the medium.

Bacterial identification: VITEK2

The Advanced Colorimetric Reading is the basis for the identification of species of microorganisms detected by the VITEK2. It is able to provide results reliable on a large number of organisms of bacteria taxonomy using a huge number of tests in card. The card for the identification is composed by a coated plastic holder and a gas-permeable film where there are 64 wells of 2 µL containing biochemicals for identification. The cards are connected by a tube to the standardized bacterial solution prepared by the operator and automatically inoculated with a bacterial suspension in saline solution inside the instrument. Once inserted into the incubator of the VITEK2, the instrument manages and controls each card. The readings for the identification are generally available in 2-8 hours.

EXPERIMENTAL METHOD IN COMPARISON

Sample purification

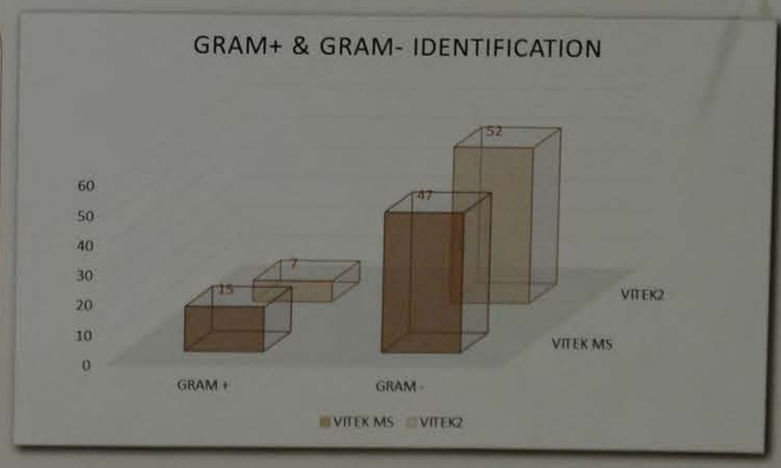
Once the screening is finished, the same vials used for ALFRED60 must undergo a first separation. 1 mL of sample is picked up from each vial and is then dispensed in 1.5 mL Safe Lock Tubes. The tubes are centrifuged a first time at 13,300 rpm for five minutes to remove the broth by invertion. To avoid interferences during the reading with MALDI-TOF, the pellets are washed with 1 mL of water for injections. Then, the pellets are resuspended by a vortex and the samples are submitted by a second centrifuged at the same speed.

Bacterial identification: VITEKMS

Once the samples are purified, the pellets are dispensed by a 1µL handle on a VITEKMS plate. A sample at a time is dispensed from the test tube and then spread in three adjacent wells in order to do an empirical dilution. When the pellet is dried, it will be possible to add 1µL of CHCA (α-cyano-4-hydroxycinnamic-acid) matrix to crystallize the sample. VITEKMS is a mass spectrometer using the MALDI-TOF tecnique. This technology provides a result in a few minutes, shown as a mass spectrum in which the signals are given by protein-based components resulting from a partial lysis of the bacterial cell wall. From the purification to the results it takes less than half an hour, depending on the number of the samples.

RESULTS 1.1

For two months an amount of 236 samples has been analyzed for authenticity, 59 of which resulted positive at ALFRED60. If compared, the results between the two different instruments for identification VITEK2 and VITEKMS almost overlap: for example, GRAM+ and GRAM- identification looks similar. VITEKMS is more accurate than VITEK2 in terms of giving results of GRAM+: 15 instead of 7 (T Test: $p<0.05$). Gram+ are difficult to culture on agar medium: it often takes more than 24 hour of incubation to get a minimum of colonies for the identification and subsequent antibiogram.





RESULTS 1.2

As far as GRAM- concern, E. coli is the most representative bacteria having been found positive in 40 samples with VITEK2 and 38 in mass spectrometry. E. Coli is a native bacteria in human intestine: the risk of infection is high because of the diapers and parents can find it difficult to clean the babies. VITEKMS results more sensible in the identification of Gram+ Enterococcus spp, bacteria also found naturally in the intestine. Some negative urines have been chosen randomly to avoid the presence of false positive samples. About Peptoniphylus Asaccarolyticus: it has been found only with VITEKMS but this technology provides a large database of spectra and the one in analysis is similar to the E. Coli one.

CONCLUSIONS

The comparison between the two technologies has been found successfully congruent. The most representative infectant in both the instruments is E. Coli. As far as Gram+ concern, Enterococcus spp is better detected by MALDI-TOF technology. The evidence of this study is not only the correlation of results between VITEK2 and VITEKMS: it demonstrates as the mass spectrometry technology decreases largely the TAT, at least for the identification of the bacteria. VITEK2 uses plated live colonies: it requires 3 hours for the screening plus 24 hours for the incubation on plate plus 9 to 15 hours for the identification and antibiogram. It means 48 to 72 hours to have a result and a therapy for the child. With this study it is demonstrated that analyzing the pellet obtained from the screening it is possible to identify the bacteria in less than 5 hours from the arrival of the sample in the laboratory. It would be possible to dose the pediatric patient with the best antibiotic 48 hours ahead without a hospital admission.

Microbiology
PA-19

Microbiology
PA-20

Higher Results of Group B Streptococcus Screening among Pregnant Women.

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Shiu Fang Chiu[1], Hui Chen Wang[1]

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臺北市立聯合醫院 TAIPEI CITY HOSPITAL

Background : *Streptococcus agalactiae* (Group B streptococcus, GBS) are common bacteria which are often found in the vagina, rectum or urinary bladder. GBS infection has significantly evolved in the high risk of severe illnesses and death during vaginal delivery. Especially in newborn infants, which can cause neonatal sepsis, neonatal meningitis, pneumonia and neonatal death or neurological sequela.

Methods : To screen for GBS during the third trimester, which is the 35th to 37th weeks of pregnant women, a swab is typically obtained from the lower vagina and rectum. The identifications of GBS by appearing the β-hemolysis on CNA agar, 3% Catalase-negative result and the arrowhead area of CAMP test.

If it shows β-hemolysis with no arrowhead area or γ-hemolysis on CAMP test, but still suspects might be Group B streptococcus. Then inoculated on Bile Esculin Agar (BEA) slant and 6.5% NaCl agar slant. The female vagina discharge, male urethral discharge and urine specimen are selected as parities to compare with pregnant women.

Results : As shown below, the latest estimates generated over the last two years (2013-2015) report that approximately 1228 of the 5731 pregnant women (21.43%) were examined with culture-positive for Group B Streptococcus by prenatal vagina swab specimen. The survey indicates that GBS infection possibility in pregnant women is significantly higher than females with vagina discharge (226/1478, 15.29 %), males with urethral discharge (58/1478, 3.92 %), the urine and foley specimen (1355/58380, 2.32 %). The antibiotic of Clindamycin and Erythromycin have the highly and increasingly resistant results (CM: 66.25%, EM: 59.54%) during recent years.

Conclusions : Higher isolation frequency of Group B Streptococcus among pregnant women is emerged as the leading cause of baby at risk of developing GBS disease. However, the GBS diagnosis provided by healthcare can monitor a healthy pregnancy with effective treatments to reduce the newborn morbidity and mortality.



Figure A and B show the negative and positive results on BEA slant & 6.5 % NaCl agar slant. Group B Streptococcus shows the negative reactions of the original color. *Enterococcus* shows the positive reactions of dark and yellow pigmentations.

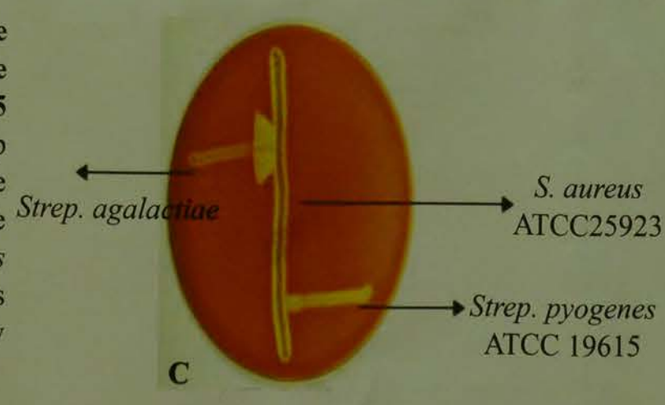


Figure C shows the CAMP test of Group B Streptococcus. It is a positive CAMP test indicated by the formation of an arrowhead area where the *Streptococcus agalactiae* meets the *Staphylococcus aureus* ATCC25923. *Streptococcus pyogenes* ATCC 19615 is for the negative control and no arrowhead area was fond .

Dates / Specimen	June 2013 - June 2014			June 2014 - June 2015		
	Total, n	GBS, n	Positive rate, %	Total, n	GBS, n	Positive rate, %
Prenatal vagina swab [a]	2787	632	22.68 %	2944	596	20.24 %
Vagina discharge [a]	770	114	14.8 %	708	112	15.8 %
Urethral discharge [a]	770	40	5.2 %	708	18	2.5 %
Urine Culture [a]	23725	655	2.76 %	23919	622	2.60 %
Foley catheter [a]	5146	41	0.8 %	5590	37	0.7 %

Table 1 shows the different specimen of GBS isolates from June 2013 to June 2015. It appears that the prenatal vagina swab from pregnant women show significantly higher than the female vagina discharge ($p < 0.1$) 、 male urethral discharge ($p < 0.05$) 、 urine culture ($p < 0.05$) and Foley catheter ($p < 0.05$) .

a) Source : Taipei City Hospital

Dates / Antibiotic	June 2013 - June 2014			June 2014 - June 2015		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Penicillin G	100 %	0	0	100 %	0	0
Vancomycin	100 %	0	0	100 %	0	0
Ampicillin	100 %	0	0	100 %	0	0
Clindamycin	26.65 %	8.06 %	65.29 %	32.39 %	1.37 %	66.25 %
Erythromycin	32.37 %	9.69 %	57.94 %	37.09 %	3.37 %	59.54 %

Table 2 shows the antibiotic susceptibilities of GBS isolates from June 2013 to June 2015. It appears that the antibiotics of Penicillin G, Vancomycin and Ampicillin are all susceptible (100%). But table 2 also shows the increasing resistant results of both Clindamycin (65.29% in 2013-2014; 66.25% in 2014-2015) and Erythromycin (57.94% in 2013-2014; 59.54% in 2014-2015).

Microbiology

PA-27

奇美醫療財團法人 奇美醫院
Chi Mei Medical Center

Compassion Accountability Effectiveness

PA-27

Evaluation of a fast diagnostic chip method applied in identification of *Mycobacterium tuberculosis*

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[3]Department of Nursing, Yuh-Ing Junior College of Health Care & Management, Kaohsiung City, Taiwan

Background

1. *Mycobacterium tuberculosis* (TB) can result in nosocomial outbreak, especially delayed diagnosis.
2. AFS (acid-fast stain) has a lower detectable rate of TB and cannot differentiate between TB and *non-tuberculosis Mycobacterium* (NTM).
3. TCM(traditional culture method) is regarded as the golden standard of identifying TB, but it is a time-consuming method.
4. The aim of this study was to evaluate the role of identifying TB by a biochip method (Dr. Chip).

Materials and Methods

1. This was a retrospective study from January to December 2013.
2. All clinical samples sent for identifying TB were enrolled.
3. Three methods were used for identifying TB, including Dr.Chip (Fig.).

Table 1. Comparison of Biochip (Dr. Chip) with TCM and ASF for detection of Mycobacterium tuberculosis (MT)and *non-tuberculosis Mycobacterium* (NTM).

		TCM(+)/AFS(+)	TCM(+)/AFS(-)	TCM(-)/AFS(+)	Total
Dr. Chip	MT	267	127	2	396
	NTM	154	319	37	510

Results

1. A total of 6819 samples, mostly obtained from sputum, were enrolled.
2. The detectable rate of TB was 5.8 % (n = 396), 5.8 % (n = 394), and 3.9% (n = 269) by using Dr. Chip, TCM and AFS, respectively. (Table 1).
3. 510 (7.5%) and 473 (6.9%) NTM were detected by Dr. Chip and TCM, respectively.
4. Dr. Chip can further distinguish the common 15species in Taiwan from NTM-positive species.

Conclusion

1. The detectable rate of TB was similar between Dr. Chip (a rapid method ,less than 24 hours) and TCM (a time-consuming method).
2. Dr. chip had a higher detectable rate and accurate rate than AFS (5.8% vs. 3.9% and 100 % vs. 58.5%, respectively).
3. The application of Dr. Chip in clinical test not only could identify MT and NTM quickly, but also further distinguish different NTM species (data not shown).
4. Hence, we consider that Dr. Chip should have an important role to identify TB, resulting in rapid diagnosis of tuberculosis and decreasing nosocomial spread.

Reference

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3. Lee MR, Cheng A, Huang YT, Liu CY, Chung KP, Wang HC, Liang SK, Liao CH, Yu CJ, Hsueh PR. Performance assessment of the DR. TBDR/NTM IVD kit for direct detection of Mycobacterium tuberculosis isolates, including rifampin-resistant isolates, and nontuberculous Mycobacteria. J ClinMicrobiol. 2012 Oct;50(10):3398-401.

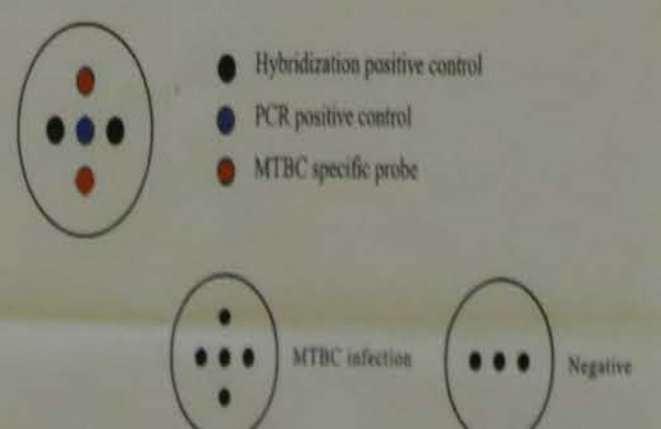


Fig. 1 The patterns that developed at the bottom of the well of the Dr. Chip.

Microbiology

PA-28

PA-28

rpoB Gene Analysis of Multidrug-resistant Mycobacterium Tuberculosis Strains Isolated in North Taiwan

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Abstract

Rifampin is an antibiotic drug for tuberculosis treatment. Rifampin resistance is most frequently conferred by mutations at 81 bp of active center of RNA polymerase encoded by *rpoB* gene. In this study, we intended to identify the mutation patterns of *rpoB* gene in multiple drug-resistant *Mycobacterium tuberculosis* (MDR-TB), and the correlation of *rpoB* gene mutation patterns with the drug resistance. Fragments in hot spot region spanning 1546-1593 nucleotides of *rpoB* gene of 91 MDR-TB strains were amplified and sequenced. MDR-TB strains were defined as mycobacteria are resistant to 0.2 μg/ml of INH and 1.0 μg/ml of rifampin. We also did second-line drug susceptibility test using the agar proportion method on 7H11 agar. The mutations mainly occurred at codon 531 (62.6%), codon 516 (11%), and codon 526 (12.1%). Only 1.1% mutation rate occurred at codons 511, 513, 522, and 533. Comparison analysis shown in Table 3, our results were identical to those of other researches in Taiwan, suggesting that the top mutation rate of *rpoB* gene was found at codon 531, the second was at codon 526 and 516. Additionally, we demonstrated the relationship of second drug resistance and *rpoB* gene hot spot region mutations. MDR-TB strains were almost fully resistant to rifabutin when mutations occurred at codon 531 in *rpoB* hot spot region. Identification of gene mutation patterns is a critical for the development of rapid molecular diagnostic tests. Mutations at 81 bp in hot spot region of *rpoB* causing rifampin resistance are well known for MTB therapy. In this study, mutations at codons 531 might be a useful diagnostic marker for second-line drug application.

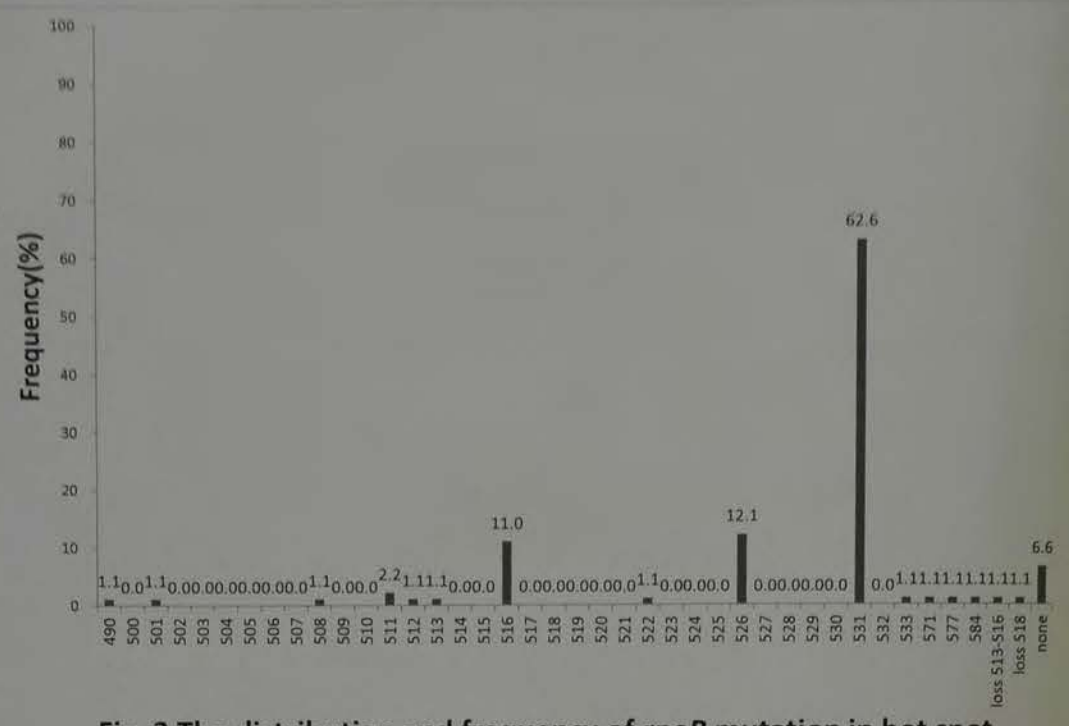


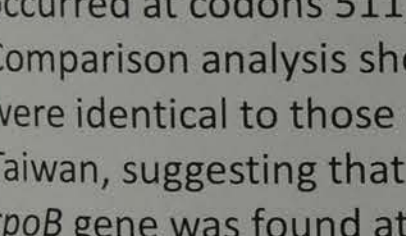
Fig. 3 The distribution and frequency of *rpoB* mutation in hot spot region codons 490 to 584.

Table 2. The location and types of *rpoB* mutations between codons 507-577.

Mutated codons	specific mutations	no.	Frequency (%)
531	TCG(Ser)→TTG(Leu)	54	59.3
531, 577	TCG(Ser) →TTG(Leu), GTG(Leu) →ATG(Met)	1	1.1
531, 584	TCG(Ser) →TTG(Leu), TTC(Phe) →TCC(Ser)	1	1.1
531, 501, 490	TCG(Ser) →TGG(Trp) , GCG(Ala) →ACG(Thr), CAG(Gln) →CGG(Arg)	1	1.1
516	GAC(Asp) →GTC(Val)	5	5.5
	GAC(Asp) →TAC(Tyr)	5	5.5
526	CAC(His) →GAC(Asp)	3	3.3
	CAC(His) →TAC(Tyr)	2	2.2
	CAC(His) →ACC(Thr)	2	2.2
	CAC(His) →CGC(Arg)	1	1.1
	CAC(His) →AAC(Asn)	1	1.1
511	CTG(Leu) →CCG(Pro)	1	1.1
513	CAA(Gln) →CCA(Pro)	1	1.1
522	TCG(Ser) →TTG(Leu)	1	1.1
533	CTG(Leu) →CCG(Pro)	1	1.1
512, 526	AGC(Ser) →GGC(Gly), CAC(His) →CGC(Arg)	1	1.1
508, 526	ACC(Thr) →CCC(Pro), CAC(His) →TAC(Tyr)	1	1.1
511, 571	CTG(Leu) →CCG(Pro), ATC(Ile) →CTC(Leu)	1	1.1
loss 1538-1546	codon 513~516 (_AATTCATGG__)	1	1.1
loss codon 518	AAC(Asn)	1	1.1
none		6	6.6
total		91	100%

Table 3 Frequency of *rpoB* mutations in rifampin-resistant M. tuberculosis isolates from different geographic regions.

Country (reference; no. of isolates)	Sample source	Frequency (%) of mutated codon								
		508	511	513	516	522	526	531	533	No mutation within hot-spot region
Italy ([7]; n=37)	MDR-TB, 1998	0	2.7	0	8.1	0	35.1	59.4	0	0
India ([8]; n= 44)	Rif-resistant isolate, 2000	2.2	6.8	2.2	4.5	0	22.7	63.6	2.2	2.2
East Asia ([9]; n = 66)	MDR-TB from China, Japan, Korea, Taiwan, 2001	0	0	6	16.7	0	10.5	51.5	3	10.5
China ([10]; n = 86)	72 Rif-resistant and 14 RIF-sensitive strains, 2002	0	2	2	4	3	40	41	2	10
Kaohsiung, Taiwan ([11]; n = 63)	rifampin -resistant isolates, 1996-1998	0	1.9	0	15.1	1.9	18.9	41.5	7.5	15.9
Taipei, Taiwan ([12]; n = 162)	MDR-TB , CDC, 1998-2003	0.6	0	4.9	8.6	2.4	20.4	49.4	3.7	9.9
TCVGH, Taiwan ([5]; n=22)	rifampin -resistant isolates, 1990 - 2011	0	0	9.1	4.5	0	4.5	68.2	9.1	4.5
this study (n=91)	**MDR-TB in Taiwan, 2010**	**1.1**	**2.2**	**1.1**	**11**	**1.1**	**12.1**	**62.6**	**1.1**	**6.6**



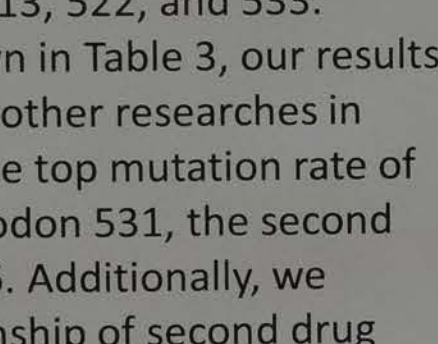


Fig.1 (a) A fragment of 407 bp PCR product located at nucleotides 1366 to 1772. (b) The percentage of various *rpoB* mutation types in MDR-TB strains

Table 1 Age and gender of 91 patients with MDR-TB strains, and relative proportion of patients with codon 531 mutation.

	codon 531		not codon 531	
	no.	%	no.	%
age				
<34	12	13.2	5	5.5
35-64	25	27.5	17	18.7
>65	20	22.0	12	13.2
sex				
male	42	46.2	19	20.9
female	15	16.5	15	16.5
total	57		34	

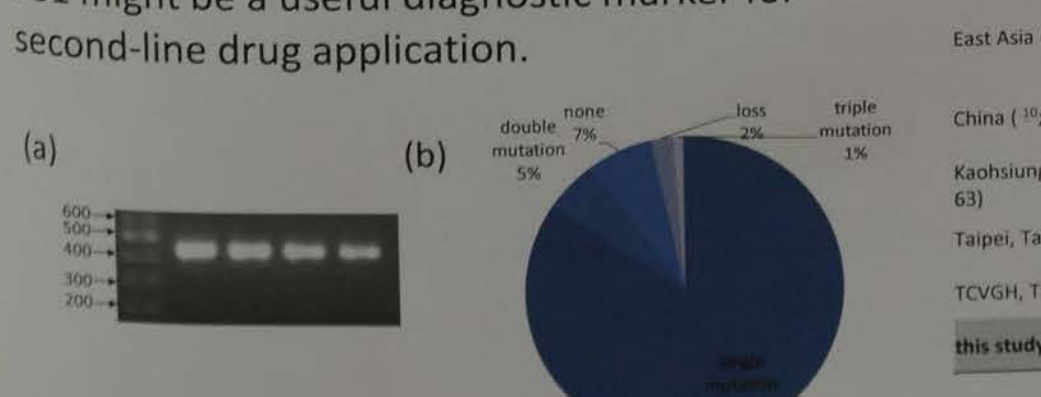
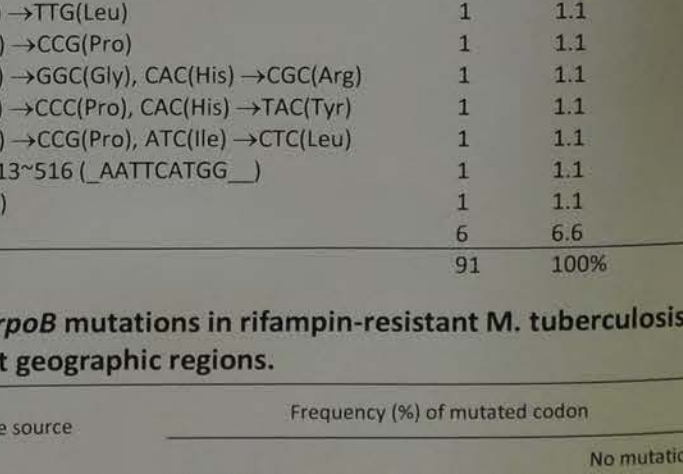


Fig.2 Types of *rpoB* mutations between codons 490 to 584.

Fig.4 The profiles of second-line drug resistance of MDR-TB with codon 531 mutation (%)



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Microbiology

PA-29

Analysis of the Antibiotic Resistance Rates and Contamination Rates of Bloodstream Infection (BSI) Cases.

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Background : Bloodstream infections (BSI) cause important morbidity and mortality worldwide. Especially in diagnostic and treatment delay among bacteremia or sepsis patients.

Methods : Total blood culture bottles were detected between January 1st to December 31th in 2015 of Taipei City Hospital of Yangming Branch. All bottles were placed into Automated BD BACTEC FX Blood Culture System. Then, the positive bottles were identified the isolates and its antibiotic susceptibility by using BD Phoenix™ 100.

Results : First, 513 positive blood culture bottles were detected in 4915 samples (513/4915, 10.5%). We yielded 550 clinically significant organisms which including 28 patients who were infected with two or over two isolates (28/513, 5.5%). The overall mortality was 19.3%. Second, 108 organisms were resistant to antimicrobial agents (108/550, 19.6%) among ER (n=38, 6.9%), OPD (n=2, 0.4%), ICU (n=18, 3.3%) and ward (n=50, 9.1%). The identified resistant-strains were MRSA (n=33, 6.0%), MRCoNS (n=20, 3.6%), MRSE (n=4, 0.7%), *ESBL*-EC (n=37,6.7%), *ESBL-KP (n=10, 1.8%), ESBL- P. mirabilis* (n=3, 0.6%). Vancomycin resistance was seen in 0.2% of *Enterococcus faecalis* isolates. Finally, the patients with BSI from blood contamination was up to 20.4% (n=112) among ER (n=70, 12.7%), ICU (n=11, 2.0%), ward (n=31, 5.6%). And there was no contaminated blood bottle among OPD.

Conclusions : Comparing with the positive rate in blood culture between 2013 (14.3%) to 2014 (12.5%). The BSI is decreased recently years. According to the data, urged that the patients with BSI are very likely to be infected with highly resistant isolates. Especially with MRSA or *ESBL*-EC. And we found that ER had higher results of resistant-strains and contamination rates rather than ICU and ward in blood culture. However, how to prevent the blood contamination and provide the correct diagnosis can monitor with effective treatments to reduce the patient morbidity and mortality.

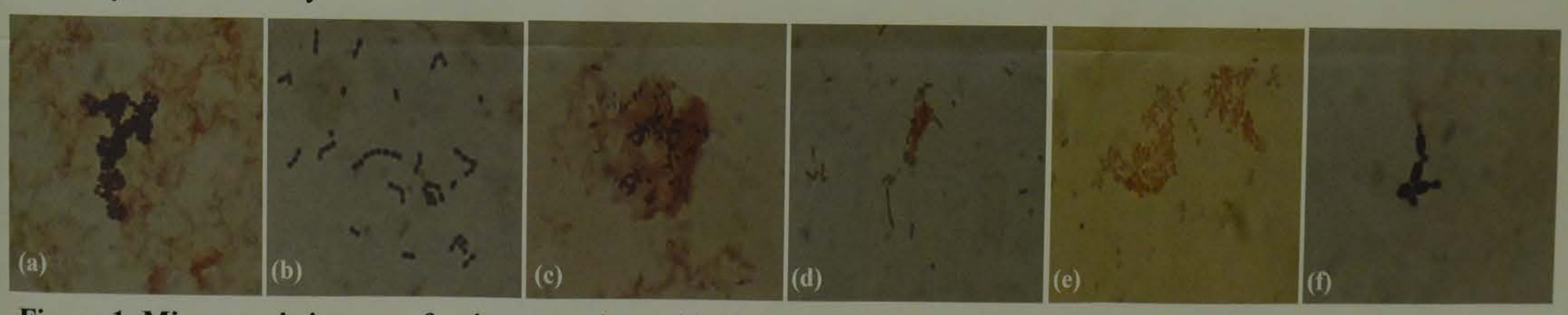


Figure 1. Microscopic images of microorganisms. (a) The Gram-positive cocci in cluster and (b) in chains. (c) Rod-shaped Gram-positive bacilli and (d) Gram-negative bacilli. (e) Gram-negative cocci. (f) Fungi by Gram staining.

Table 1. The frequency of bacteria isolated from blood cultures of patients.

	ER	OPD	ICU	Ward
	Case (%)	Case (%)	Case (%)	Case (%)
Gram-positive cocci				
S. aureus	19 (7.4)	0	7 (9.0)	31 (14.8)
S. epidermidis	6 (2.3)	0	2 (2.6)	14 (6.7)
Other CoNS	73 (28.5)	1 (20)	13 (16.7)	52 (24.9)
Enterococcus	3 (1.2)	0	8 (10.3)	6 (2.9)
Gram-negative bacilli				
E.coli	63 (24.6)	1 (20)	14 (17.9)	27 (12.9)
K. pneumoniae	25 (9.8)	2 (40)	2 (2.6)	11 (5.3)
P. mirabilis	5 (1.9)	0	4 (5.1)	6 (2.9)
Fungus	1 (0.4)	0	11 (14.1)	7 (3.3)
GBS	14 (5.5)	1 (20)	1 (1.2)	9 (4.3)
others	47 (18.4)	0	16 (20.5)	46 (22)
Total	256 (100)	5 (100)	78(100)	209(100)

Table 2. Antibiotic resistance in common bacterial groups in blood cultures.

	ER		OPD		ICU		Ward		
	case	%	case	%	case	%	case	%	p-value
Gram-positive cocci									
MRSA	9	47.4%	0	0%	6	85.7%	18	85.1%	<0.05*
MRSE	0	0%	0	0%	0	0%	4	28.6%	0.195
MRCoNS	8	10.9%	1	100%	2	15.4%	9	17.3%	<0.05*
VRE	0	0%	0	0%	0	0%	1	16.7%	0.221
Gram-negative bacilli									
ESBL-EC	17	26.9%	0	0%	7	50%	13	48.1%	<0.05*
ESBL-KP	4	16%	1	50%	1	50%	4	36.4%	<0.05*
ESBL- P. mirabilis	0	0%	0	0%	2	50%	1	16.7%	0.141

MRSA : methicilillin-resistant *Staphylococcus aureus*
MRSE : methicilillin-resistant *Staphylococcus epidermidis*
MRSE : methicilillin-resistant coagulase-negative staphylococci
VRE : Vancomycin-resistant *enterococcus*
ESBL-EC : extended-spectrum beta-lactamases- *Escherichia coli*
ESBL-KP : extended-spectrum beta-lactamases- *Klebsiella pneumoniae*
ESBL- P. mirabilis : extended-spectrum beta-lactamases- *Proteus mirabilis*

Microbiology

PA-30

MOLECULAR CHARACTERIZATION OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM SHELLFISH AND THEIR HARVESTING WATER FROM SUEZ CANAL AREA, EGYPT

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ABSTRACT

Vibrio parahaemolyticus is considered a natural pathogen of the aquatic environment. It is a leading cause of seafood-derived food poisoning throughout the world. The main objectives of this study were to determine the prevalence of contamination of shellfish by *V. parahaemolyticus* in Suez Canal area and to assess its molecular characteristics. The study included 205 samples of shellfish (82 clams, 43 mussels, and 80 shrimps) collected from Suez Canal area (78 from Port Said, 82 from Ismailia and 45 from Suez governorates). In addition, the harvesting water samples were collected from different sites. All samples were collected during the warmest season (June, July and August) through two years research period. After extraction of tissue and homogenization, enriched samples were identified by plating onto TCBS agar medium. Presumptive *V. parahaemolyticus* blue to green colonies were selected and purified and further identified by API20 and PCR techniques targeting *toxR* gene to be confirmed as *V. parahaemolyticus*. The pathogenicity of the isolates was examined by detection of Thermostable Direct Haemolysin (TDH) and related Haemolysin (TRH) genes. Results revealed that the overall prevalence of *V. parahaemolyticus* in shellfish was 19/205 (9.27%) whereas in water was 6/24 (25%). Higher contamination rate was detected in shrimp (15%), and the highest rate was in Ismailia governorate (12.2%). The detection rate of TDH and TRH genes was 21.05% and 5.26%, consequently.

OBJECTIVES

- Investigation of the prevalence of *V. **parahaemolyticus*** in shellfish and their harvesting water at Suez canal area, Egypt.
- Examining the occurrence of two genes responsible for the pathogenicity (TDH &TRH genes).
- Assessment of it's the public health significance.

MATERIAL AND METHODS

Sample collection



Clams No=82



Mussels No=43



Shrimp No=80



Africa



Northeast Egypt

Sample preparation

- All samples were collected during the warmest season (June, July and August) during each sampling year through two years research period.
- Samples were disinfected externally before being shelled on clean sterile surface using sterile forceps and scissors.

Isolation and identification of *V. parahaemolyticus*

- *V. Parahaemolyticus was isolated* according to (APHA,1992).
- The homogenized samples and water samples were enriched by incubation on BPW with 2% Nacl at 37C for 24 h.
- Enriched samples were identified by plating onto TCBS agar.
- Presumptive *V. parahaemolyticus* appeared as blue to green colonies were selected and purified on TCBS agar and were examined by biochemical tests.
- Isolates were further confirmed as *V. parahaemolyticus* by API20 and PCR techniques targeting *toxR* gene (Kim *et al.*, 1999).

PCR detection of TDH and TRH genes

- DNA extraction from enriched samples was carried out by boiling method as described by Tunung *et al.* (2011).
- TDH and TRH genes were examined by PCR techniques according to Tada et al., 1992.
- *V. parahaemolyticus* strain (ATCC 17802) was used as reference strain.

RESULTS

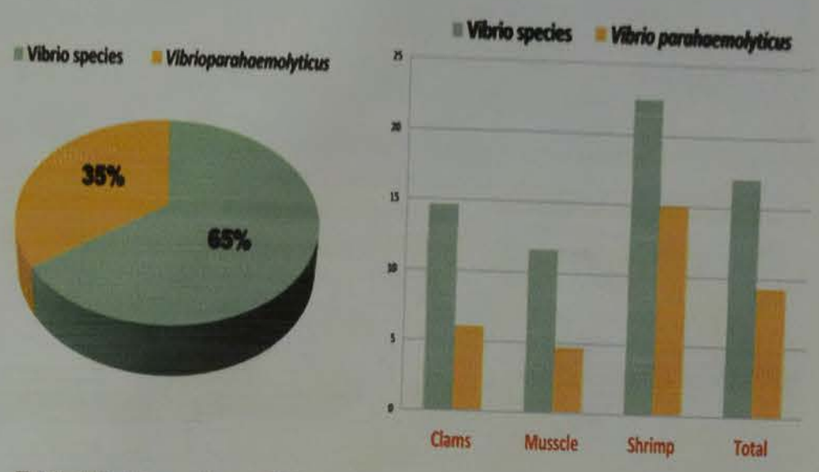


Table 1: Total prevalence of *Vibrio* species from shellfish from 3 locations.

Shellfish	Port Said	Ismailia	Suez	Total
Clams	7/28 (25%)	5/40 (12.5%)	0/14 (0%)	12/82 (14.63%)
Mussle	3/12 (25%)	2/14 (14.29%)	0/17 (0%)	5/43 (11.63%)
Shrimp	6/38 (7.89%)	9/28 (32.14%)	3/14 (21.43%)	18/80 (22.50%)
Total	16/78 (20.51%)	16/82 (19.12%)	3/45 (6.67%)	35/205 (17.07%)

Table 2: Total isolation rate of *V. parahaemolyticus* from Shellfish from 3 locations

Shellfish	Port Said	Ismailia	Suez	Total
Clams	2/28 (7.14%)	3/40 (7.5%)	0/14 (0)	5/82 (6.01%)
Mussle	1/12 (8.33%)	1/14 (7.14%)	0/17 (0)	2/43 (4.65%)
Shrimp	4/38 (10.53%)	6/28 (21.43%)	2/14 (14.29%)	12/80 (15%)
Total	7/78 (8.94)	10/82 (12.20%)	2/45 (4.44%)	19/205 (9.27%)

Prevalence of *V. parahaemolyticus* in shellfish



Prevalence of *V. parahaemolyticus* in different locations

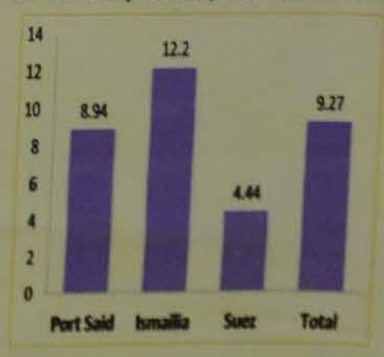
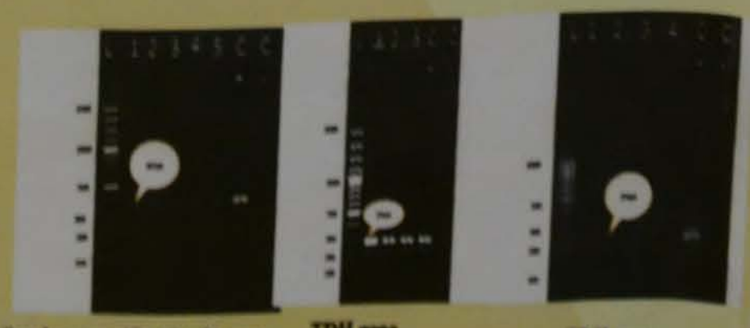


Table 3: Distribution of TDH and/or TRH *in V. parahaemolyticis* samples

Shellfish	TDH	TRH	TDH/TRH
Clams	1/5 (20%)	0/5 (0%)	0 (0%)
Mussel	0/2 (0%)	0/2 (0%)	0 (0%)
Shrimp	3/12 (25%)	1/12 (8.33%)	1/12 (8.33%)
Total	4/19 (21.05%)	1/19 (5.26%)	1/19 (5.26%)



Species – specific (ToxR) gene TDH gene TRH gene

CONCLOSIONS:

This study demonstrated the presence of potentially pathogenic *V. parahaemolyticus* in some shellfish at Suez Canal area, Egypt which may present a risk of seafood borne illness .

References:

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Microbiology

PA-31

High prevalence of Mycoplasma genitalium macrolide resistance in Oslo, Norway.

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INTRODUCTION

Mycoplasma genitalium is a sexually transmitted bacterium with the ability to cause urethritis and cervicitis. Recommended treatment for uncomplicated *M. genitalium* infection is the macrolide azithromycin, 1.5 g given over a period of 5 days. However, resistance to macrolides has been more frequently reported in many parts of the world. Macrolide resistant strains have been shown to contain base substitutions in positions 2058 and 2059 (*E. coli* numbering) in the 23S rRNA V region. A2058G and A2059G are the most common mutations reported, followed by A2058T. Second line antibiotics following azithromycin treatment failure is the fluoroquinolone moxifloxacin, but resistance towards this group of antibiotics have also been reported.

The aim of this retrospective study was to describe the prevalence of *M. genitalium* macrolide resistance from patient in the Oslo area, Norway.

METHODS

Samples

Clinical samples obtained during a two months period from patients visiting Olafiaklinikken, an open access STI clinic in Oslo, were included.

***M. genitalium* in house real-time PCR**

MagnaPure 96 (Roche) was used for DNA isolation and LC480 (Roche) for the PCR. The primers targeting the MgPa adhesine gene (1).

Identification of *M. genitalium* macrolide resistance by Sanger sequencing

DNA from *M. genitalium* PCR positive samples were amplified by PCR using primers targeting *M. genitalium* 23S rRNA gene, Mg23S-1992F and Mg23S-2138R, as described by Jensen JS et al (2). The amplified fragments were sequenced using ABI Big Dye terminator sequencing kit and ABI 3130 xl Genetic Analyzer (Life technologies). The sequences were analyzed and compared with the wild-type (WT) sequence using Sequencher.

RESULTS

A total of 3525 samples were analyzed by our in house *M. genitalium* real-time PCR, of which 155 (4.4 %) were positive. Ninety eight of the 155 *M. genitalium* positive samples were analyzed for macrolide resistance-associated mutations, and mutations in position 2058 and 2059 were found in 63 samples (64 %), (Fig. 1). The predominant mutations identified were A2059G (36.7 %) and A2058G (23.5 %), followed by A2058T (3.1%). The base substitutions A2058C and A2059C was not detected but A2059C strains have been detected in samples analyzed after the study period. One sample contained a mix of A2058G and WT sequence (Fig. 2). Only the wt strain was detected in a sample from this patient submitted one month earlier. The four macrolide resistant negative samples were weak positive in the primary *M. genitalium* PCR screening (Ct-value >40).

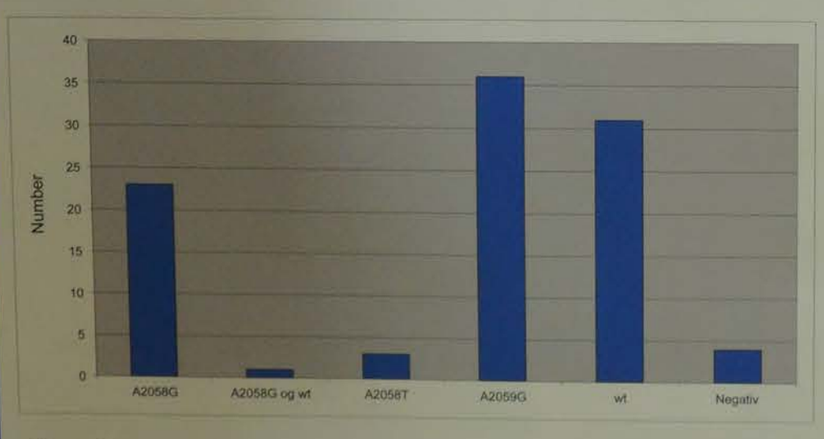


Figure 1.
Distribution of macrolide resistant and wt *M. genitalium* strains in the area of Oslo, Norway. (N = 98)



Figure 2.
Two samples taken a month apart from the same patient. Sequencing indicates a switch from wt to a mix of wt and A2058G mutation.

CONCLUSION

We have detected macrolide resistance-associated mutations in *M. genitalium* at a rate of 64 %.
Although the sample size is small, this study indicates that macrolide resistant *M. genitalium* strains occur with a high frequency in the Oslo area. This confirms the importance of a test of cure to detect failure of standard treatment. Otherwise, the needs for prospective surveillance of macrolide resistance to guide treatment of patients and reduce the use of antibiotic must be emphasized.

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2. Jensen JS et al: Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008 Dec 15;47(12): 1546-53.

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UiO : University of Oslo

Microbiology

PA-32

Norwegian Institute of Public Health

Salmonella identification by MALDI-TOF MS

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Introduction

Biochemical and serological methods are traditionally used to identify *Salmonella* from clinical samples. The National Reference Laboratory for Enteropathogenic Bacteria; Norwegian Institute of Public Health wanted to examine if MALDI-TOF MS (Bruker Daltonik GmbH) could replace biochemical tests in the identification of *Salmonella* to genus level prior to serological testing for final identification. At the same time, we wanted to examine whether various growth media would influence the results obtained using this method.

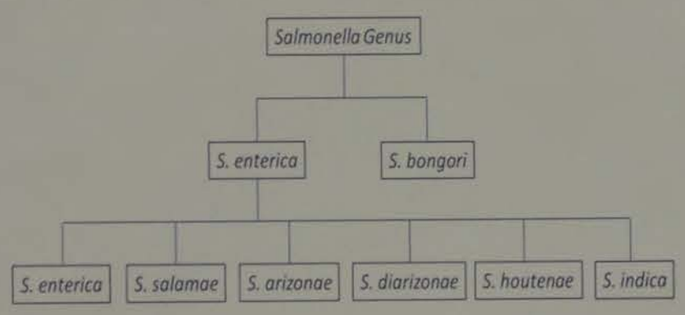


Fig. 1. Overview of taxonomic classification of *Salmonella.*



Fig. 2. Example of biochemical tests.

Material and Methods

A total of 101 *Salmonella* isolates from our national strain collection were analysed:

- Species *S. enterica* (including all six subspecies:
  - *S. enterica* (50)
  - *S. salamae* (11)
  - *S. arizonae* (6)
  - *S. diarizonae* (13)
  - *S. houtenae* (10)
  - *S. indica* (1)
- Species *S. bongori* (10)



Fig. 3. *Salmonella* grown on Columbia blood- ,lactose- and nutrient agar.

Morphological and phenotypical deviant strains from *S. enterica* ssp. *enterica* were among the included isolates. Each isolate had previously been identified using standard biochemical and serological methods (Fig. 2 and Table 1).

Bacteria were grown on lactose-, nutrient- and Columbia blood-agar, which are traditionally used in our laboratory (Fig. 3), and subsequently analysed using Microflex LT MALDI-TOF MS with MALDI biotyper 3.1 software (Fig. 4). Each isolate was prepared in duplicate using the smear method described by the manufacturer. The parallell with the lowest score was excluded.



Fig. 4. Microflex LT MALDI-TOF MS (Bruker Daltonik GmbH).

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
Characters							
Dulcitol	+	+	–	–	–	d	+
ONPG (2 h)	–	–	+	+	–	d	+
Malonate	–	+	+	+	–	–	–
Gelatinase	–	+	+	+	+	+	–
Sorbitol	+	+	+	+	+	–	+
Growth with KCN	–	–	–	–	+	–	+
L(+)-tartrate[a]	+	–	–	–	–	–	–
Galacturonate	–	+	–	+	+	+	+
γ-glutamyltransferase	+(*)	+	–	+	+	+	+
ß-glucuronidase	d	d	–	+	–	d	–
Mucate	+	+	+	– (70%)	–	+	+
Salicine	–	–	–	–	+	–	–
Lactose	–	–	– (75%)	+ (75%)	–	d	–
Lysed by phage O1	+	+	–	+	–	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

(a) = *d*-tartrate.
(*) = Typhimurium d, Dublin –.
\+ = 90 % or more positive reactions.
– = 90 % or more negative reactions.
d = different reactions given by different serovars.

Table 1. Differential characters of *Salmonella* species and subspecies (Grimont and Weill, 2007).

Results

The results showed acceptable scores (≥2.0) for all *Salmonella* strains to genus level except for *S. enterica* ssp. *Houtenae* (Fig. 5*)*. This subspecies was incorrectly identified in some duplicate tests and generally showed lower scores (<2.0). The score was ≥2.0 for 295 out of 303 analyses independent of growth media.

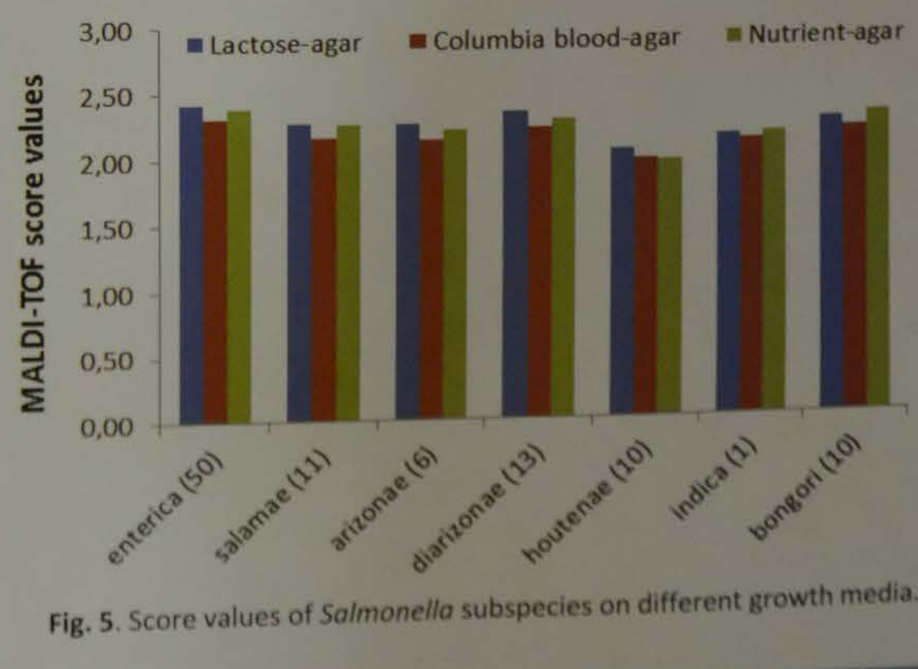


Fig. 5. Score values of *Salmonella* subspecies on different growth media.

Conclusions

- MALDI-TOF MS can replace biochemical methods for detection of *Salmonella* to genus level. The method did, however, have difficulties in identifying *S.houtenae*, but infections caused by this microbe are very rare in humans.
- Serological tests are still necessary to identify serotypes. Additional biochemical testing must be performed if different subspecies have equal antigenic formulae.
- The three growth media utilized are all equally suitable for use in the identification of *Salmonella* with MALDI-TOF MS.

Microbiology

PA-33

Microbiology

PA-33



Setted Up A New Method For Detection Of *Clostridium Difficile* Ribotypes In Stool Samples

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Introduction

Clostridia (members of the genus Clostridium) are anaerobic, motile bacteria, ubiquitous in nature, and especially prevalent in soil. Under the microscope, they appear as long, irregular (often drumstick- or spindle-shaped) cells with a bulge at their terminal ends. Under Gram staining, C. difficile cells are Gram-positive and show optimum growth on blood agar at human body temperatures in the absence of oxygen. When stressed, the bacteria produce spores that are able to tolerate extreme conditions that the active bacteria cannot tolerate.

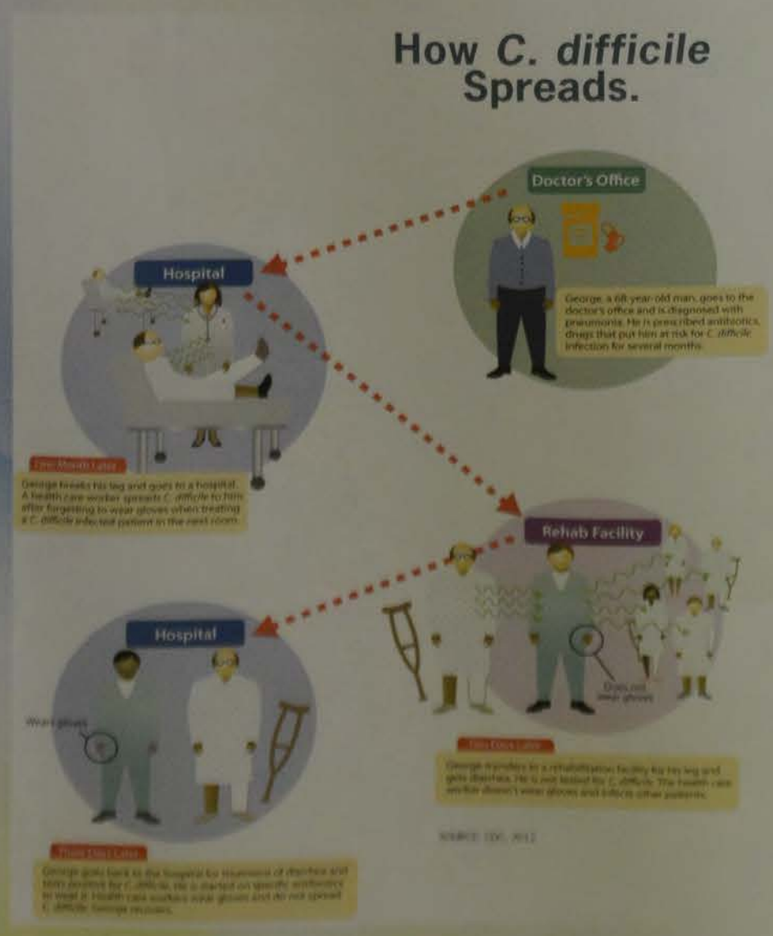




C. difficile may become established in the human colon; it is present in 2–5% of the adult population.Sometimes antibiotic therapy for various infections has the adverse effect of disrupting the normal balance of the gut flora, in which case C. difficile may opportunistically dominate, causing Clostridium difficile colitis.

Clostridium difficile infections represent a significant burden on the health care system. Although many infections are sporadic, nosocomial transmission is still important and outbreaks are a constant threat in the hospital environment. The ability to detect such outbreaks quickly is critical to infection control.

Several typing techniques, all of them based on having a pure culture of the organism, have been described for *C. difficile* . Pulsed-field gel electrophoresis (PFGE) and PCR ribotyping are the methods of choice in North America and in Europe, respectively. Three variations of PCR ribotyping have been described, two of them differing in the primers used, and while two use traditional agarose gel-based analysis , the third uses capillary gel electrophoresis-based analysis of the results . Here, we used a new method of PCR ribotyping that can be used for detection of C. difficile ribotypes directly
in stool samples.



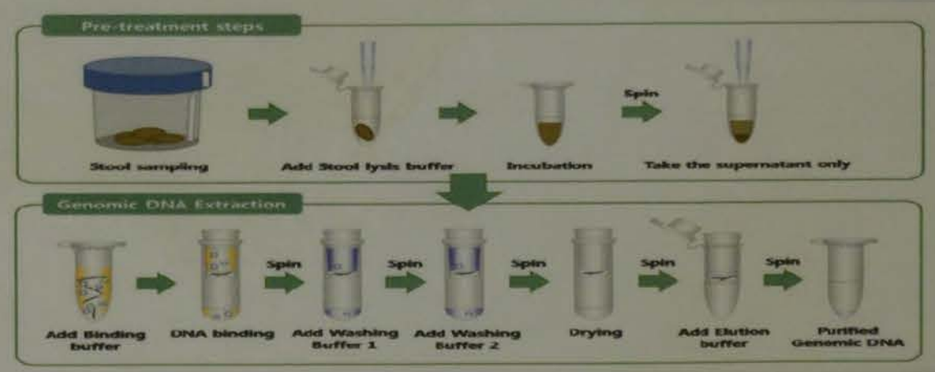
C. difficile is transmitted from person to person by the fecal-oral route. The organism forms heat-resistant spores that are not killed by alcohol-based hand cleansers or routine surface cleaning. Thus, these spores survive in clinical environments for long periods. Because of this, the bacteria may be cultured from almost any surface. Once spores are ingested, their acid-resistance allows them to pass through the stomach unscathed. Upon exposure to bile acids, they germinate and multiply into vegetative cells in the colon.

Material and method

In this study, we collected 60 samples. Then we done the GDH test , if the result was positive , we started this examination.



A Nautia stool DNA isolation min ikit was used to extract total DNA from stool samples, and 5 ul was used as template DNA for PCR. The ISRs were amplified in a final volume of 50 ul containing 5 ul of DNA, 50 pmol of each primer, 1.5 mM MgCl2, 0.1 mg/ml bovine serum albumin (BSA), and 1.25 U of Taq DNA polymerase. The amplification conditions were as follows: an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 1 min at 95°C for denaturation, 1 min at 57°C for annealing, and 1 min at 72°C for elongation, plus 10 min at 72°C for final elongation. Amplification products were concentrated to a final volume of 25 ul by heating at 75°C for 45 min before electrophoresis in 3% agarose gel for 5 h at 2.5 V/cm.

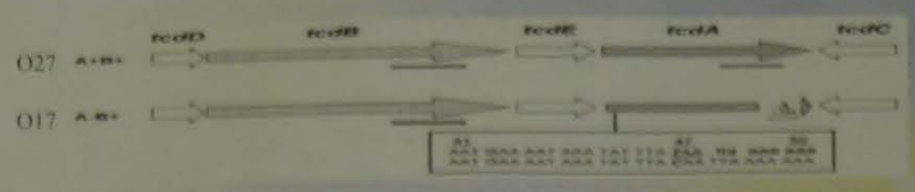


For direct PCR ribotyping from stool samples, we designed new primers located partially within the C. difficile 16S-23S rRNA intergenic spacer region (ISR) and partially within the 16S (forward primer) and 23S (reverse primer) rRNA genes. New primers were defined on the basis of DNA sequences of the ISRs of 29 different PCR ribotypes(see below). These primers resulted in increased specificity for C. difficile and could be used for direct typing from the stool sample.

NO1	primer	sequence
1	16s RNA forward 23s RNA reverse	5' GCTGGATCACCTCCTTTCTAAG 5' TGACCAGTTAAAAAGGTTTGATAGATT
2	16s RNA forward 23s RNA reverse	5'GTTTTCCCAGTCACGAC 5'AACAGCTATGACCATG
3	16s RNA forward 23s RNA reverse	5'CTGGGGTGAAGTCGTAACAAGG 5'GCGCCCTTTGTAGCTTGACC
4	16s RNA forward 23s RNA reverse	5'GTGCGGCTGGATCACCTCCT 5'CCCTGCACCCTTAATAACTTGACC

Result

1. In this study , we found that 5' GCTGGATCACCTCCTTTCTAAG(16s RNA forward) and 5' TGACCAGTTAAAAAGGTTTGATAGATT(23s RNA reverse) was the best primer for Clostridium difficile Ribotypes.
2. According to the data , all of the GDH positive result can detected by 16s-23s primer.
3. We used the blastn database ,all of the positive result show the C.difficile belonged 017 strains.
4. Most of C.difficile O17 without tcdA gene, that's to say, we don't have to check the toxin A in Taiwan.



Summary

Direct PCR ribotyping gives the information on the presence and type of *C. difficile* within hours, in contrast to standard culture-dependent methods where typing results can be obtained only after 3 days or more (48 h for culture and 1 day for PCR ribotyping). Direct PCR ribotyping on DNA isolated from stool samples is convenient, rapid, and useful for the detection of specific types of *C. difficile* in fecal samples.

Microbiology

PA-34

Molecular characteristics of *Clostridium difficile* isolated in Kobe, Japan

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Introduction

Toxigenic *Clostridium difficile* is a cause of antibiotic-associated diarrhea and other intestinal diseases. *C. difficile* infection is a great concern in hospitals and other healthcare facilities, because *C. difficile* forms spores which can survive on surfaces for prolonged periods and they are easily transmitted from hands to hands. *C. difficile* produces two main toxins, which are an enterotoxin A (*tcdA*) and a cytotoxin B (*tcdB*). A third toxin, *C. difficile* toxin (CDT) called binary toxin is composed of enzymatic (*cdtA*) and cell binding/translocation (*cdtB*). CDT-producing *C. difficile* is frequently associated with increased severe *C. difficile* infection. In those CDT-producing *C. difficile* strains, some kinds of the hyper-virulent strains, having mutations in *tcdC* as a putative negative regulator of *tcdA* and *tcdB*, have caused outbreaks worldwide. Several kinds of mutations in *tcdC* are reported, single base-pair deletion at position 117, 18-bp deletion at positions 330-347, and a C→T transition at position 184. (1) Our purpose of this study is to investigate the molecular characteristics of toxigenic, mainly CDT-producing, *C. difficile* strains isolated in Kobe, Japan.

(1)Persson S, et al. Clin Microbiol Infect 14(11):1057-1064, 2008

Materials and Methods

☐Clinical samples

A total of 142 isolates of *C. difficile* were obtained from Kobe University Hospital between April 2012 and December 2014. Strains were identified with the C. DIFF QUIK CHEK COMPLETE test (Alere Medical, Tokyo, Japan).

☐Culture condition

C. difficile was isolated on cycloserine-cefoxitin-mannitol agar (Nissui Pharmaceutical Co Ltd., Tokyo, Japan) from stool specimens, which were treated with alcohol for spore selection following anaerobic incubation at 35ºC for 48 to 72 hours. And to enrich, a single colony was cultured anaerobically in 2-YT Broth (Life Technologies).

☐DNA extraction

The template DNA was extracted by using an Ultra Clean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc.)

☐PCR and genotyping

Isolated strains were identified by 16S rRNA gene sequencing of *C. difficile* using primers PS13 (5'-GGAGGCAGCAGTGG-GGAATA-3') and PS14 (5'-TGACGGGCGGTGTGTACAAG-3').(1) In the identified strains, we detected the presence of *tcdA*, *tcdB*, *cdtA*, *cdtB* and *tcdC*. PCR was conducted by using TaKaRa Ex Taq (TaKaRa Bio Inc., Shiga, Japan). The PCR mixtures contained 0.02 units 1 x TaKaRa Taq buffer, 0.2 mM dNTP Mixture and 0.4 μM each primer. We also determined the *tcdC* sequence of the strains which had both *tcdA* and *tcdB*, or either *tcdA* or *tcdB* by sequencing analysis.

☐rep-PCR

The clonal relationship of CDT-producing strains was explored by repetitive-sequence-based PCR (rep-PCR). rep-PCR was conducted by using DiversiLab DNA Fingerprinting kit (bioMerieux, Grenoble, France). The PCR products were separated with Agilent Bioanalyzer 2100 (Agilent Technologies, Inc., Palo Alto, CA). Analysis was performed with DiversiLab software (ver.3.3.40) using the Pearson Correlation method. Isolates with 95% homology were similarity.

Results

☐Toxigenic types

Among the isolates, 99 (69.7%) were toxigenic **(Table 1)**. In the toxigenic strains, 4 (4.0%) were CDT-producing strains (*tcdA*+/ *tcdB*+/ *cdtA*+/ *cdtB*+), and 95 (95.9%) were non CDT-producing strains (81 strains: *tcdA*+/ *tcdB*+/ *cdtA*-/ *cdtB*-; 14 strains: *tcdA*-/ *tcdB*+/ *cdtA*-/ *cdtB*-).

Table 1. Toxin genotypes of *C. difficile* strains.

tcdA	*tcdB*	*cdtA/cdtB*	No. of strains(%)
+	+	+/+	4 (4.0%)
+	+	-/-	81 (81.8%)
-	+	-/-	14 (14.1%)

Table 2 showed the types of mutation in *tcdC* of toxigenic *C. difficile* strains. One CDT-producing strain had a single base-pair deletion at nucleotide position 117 and 18-bp deletion at positions 330-347 in *tcdC*. Two CDT-producing strains had a C→T transition at position 184 in *tcdC*. One non CDT-producing strain (*tcdA*+/ *tcdB*+/ *cdtA*-/ *cdtB*-) had a 18-bp deletion at positions 330-347 in *tcdC*.

Table 2. The types of mutation in *tcdC* of toxigenic *C. difficile* strains.

tcdA/tcdB	*cdtA/cdtB*	tcdC a single-pair deletion at position 117	tcdC 18bp deletion at position 330-347	tcdC a C→T transition at position 184	No. of strains
+/+	+/+	+	+	-	1
+/+	+/+	-	-	+	2
+/+	+/+	-	-	-	1
+/+	-/-	-	+	-	1
+/+	-/-	-	-	-	80
-/+	-/-	-	-	-	14

☐rep-PCR

Four CDT-producing strains showed <95% similarity related to *tcdC* mutation by rep-PCR **(Fig. 1)**. The result revealed a variability of CDT-producing *C. difficile* in Kobe.

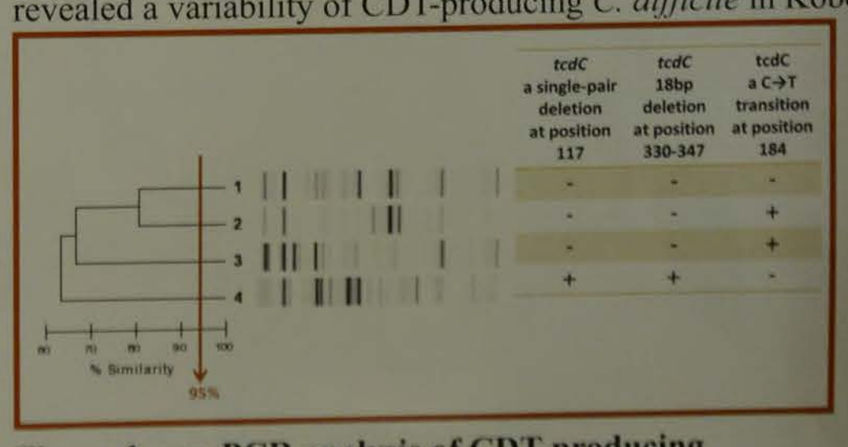


Figure 1. rep-PCR analysis of CDT-producing *C. difficile* strains in relation with the mutation in *tcdC*.

Conclusions

We found CDT-producing strains in *C. difficile* isolates having mutations in *tcdC*. The rep-PCR results revealed no clonal proximity in these CDT-producing strains. Our findings suggest the trend of these CDT-producing strains spreading in Kobe, Japan.

Microbiology

PA-35

Characterization and genetic analysis of a menadione-dependent small-colony variant of methicillin-resistant *Staphylococcus aureus*

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Introduction

Small-colony variants (SCVs) are mutant strains, which show atypical colony morphology, and require specific nutrient factors for growth. These strains sometimes cause persistent or recurrent infections. SCVs of *Staphylococcus aureus* may be misidentified or overlooked by routine microbiological methods.

Menadione-dependent SCVs often have mutations in genes related to menaquinone biosynthesis. Menaquinone is known as component of the electron-transport system. Menadione is a synthetic form of vitamin K. It is suggested that there are nine enzymes including MenA, MenB, MenC, MenD, MenE, MenF, MenH, MenI, and UbiE related to biosynthesis menaquinone (Fig. 1).

We report the characteristics and genetic analysis of a menadione-dependent SCV of methicillin-resistant *S. aureus* (MRSA) isolated from a clinical sample.

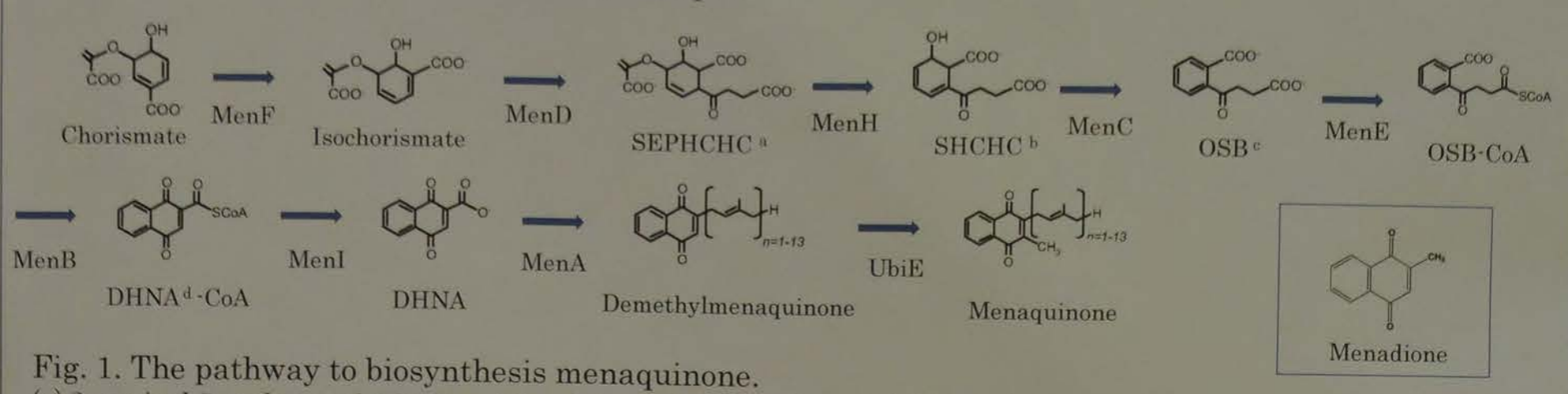


Fig. 1. The pathway to biosynthesis menaquinone.
(a) 2-succinyl-5-enoloyruvyl-6-hydroxyl-3-cyclohxene-1-carboxylic acid; (b) (1R,6R)-2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid; (c) 2-succinylbenzoate; (d) 1,4-dihydroxy-2-naphthoate.

Materials and Methods

Bacterial isolate. A *S. aureus* SCV isolated from a patient at the Shinshu University Hospital was used in this study. The SCV isolate showed atypical colony morphology on blood sheep and BTB agar (Fig. 2).

Identification and antimicrobial susceptibility tests of the SCV isolate. These examinations was performed by API Staph (Sysmex-biomerieux) and Microscan Pos MIC 3.3J panel (Beckman coulter) tests, respectively.

Detection of *mecA*. The *mecA* was detected by PCR.

Auxotrophy assay. Auxotrophic test was executed on BTB agar using three standard disks impregnated with 50 μg of hemin, 10 μg of thymidine, and 1 μg of menadione.

Sequence analysis of menaquinone biosynthesis-related genes. DNA sequences of *menA*, *menB*, *menC*, *menD*, *menE*, *menF*, *menH*, and *ubiE*, all related to menaquinone biosynthesis, were sequenced by the Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems). The sequence data were compared with those available in the International Nucleotide Sequence Database by BLAST algorithm.

Results

The SCV isolate was identified as *S. aureus* by the API Staph test, and resistant to cefoxitin, arbekacin, and so on (Table 1).

PCR demonstrated that the SCV isolate possessed *mecA*.

The auxotrophic test showed its dependence on menadione for growth (Fig. 3, 4). The SCV isolate had no genetic mutation in eight genes including *menA, menB, menC, menD, menE, menF, menH,* and *ubiE*.



Fig. 2. Colony morohology of the SCV isolate (A) and *S. aureus* ATCC29213 (B) on sheep blood agar and BTB agar after 24 hour incubation at 35℃.



Fig. 3. PCR for *mecA*.
Lane 1, size marker;
Lane 2, negative control;
Lane 3, positive control;
Lane 4, the SCV isolate.



Fig. 4. Confirmation of menadione auxotrophy on BTB agar after 24 hour incubation at 35℃.

Table 1. Identification and antimicrobial susceptibility tests of the SCV isolate

API Staph		
Profile number	Identification	%
6736153	*S. aureus*	97.7

Microscan Pos MIC 3.3J panel	
Antimicrobial	MIC (μg/ml)
Oxacillin	>2
Cefoxitin	>4
Gentamicin	>8
Arbekacin	>8
Erythromycin	>4
Clindamycin	>2
Minocycline	>8
Levofloxacin	>4
Sulfamethoxazole-trimethoprim	≦0.5/9.5
Fosfomycin	>16
Vancomycin	1
Teicoplanin	≦2
Linezolid	1

Discussion

The SCV isolate in this study was a menadione-dependent SCV of MRSA.

The SCV isolate was resistant to arbekacin. The uptake of aminoglycosides is related to membrane potential. However, defective electron-transport systems cause failure of membrane potential. Menadione-dependent SCVs have defective electron-transport systems that result in the decreased uptake of aminoglycosides, thereby causing increased antibiotic resistance[1].

The SCV isolate had no mutation in the eight genes associated with menaquinone biosynthesis. A mutation in the promoter region may cause loss of expression of these genes. Further genetic analysis is necessary to reveal the cause of its dependence on menadione.

Conclusion

The SCV isolate was determined to be MRSA by biochemical test and Microscan Pos MIC 3.3J panel. The SCV isolate required menadione for growth, but it had no mutation in the menaquinone biosynthesis-related genes.

Reference 1) Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol. 2006 Apr;4(4):295-305.

Microbiology

PA-36

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In-house Protocol for Clinical Sputum Specimen Inoculation by Using Previ Isola® Automated System

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Abstract

Lab Protocol Standardization is the approach to manage laboratory services to be more efficient and cost effective with reliable performance. With the fashion invention, clinical labs now can apply automated specimen inoculating system to standardize lab routine inoculation protocol for liquid-base specimen. Sputum specimen, however, due to its nature is challenged for establishing auto-inoculating protocol. In our hospital, sputum takes 50% of clinical microbiology lab sample size, and the sample size is increasing yearly. In this study, we evaluate Previ Isola® (bioMérieux, Craponne, France) with a special protocol for sputum specimen inoculation.

185 clinical sputum specimens were collected for the study (2015/08/05-2015/09/05). Specimens were first manually inoculated on to culture media; the remained specimens were liquefied by equal volume of 5% N-acetyl-L-cysteine(NALC) in pH 7.4 PBS buffer. After vortex and 30 minutes standing at room temperature, 10ul liquefied sample were applied for Previ Isola® plate streaking. The variety of colonies, the quality of colony growth, and the possibility to isolate single colony from the two inoculation methodologies were investigated and compared after 24 hours incubation.

The positive and negative specimen culture concordance for the two inoculation methodologies was 98.9 % (183 /185). 2 inconsistent results were from trace sample: one positive in automated method and one in manual method). In average, lab technician needs to re-isolate 5% of manual inoculated sputum specimens due to single colonies were hardly found after incubation. In the study, the culture plate prepared by automated streaking system revealed single colonies in more specimens than manual procedure. The new protocol could help to speed up the time to result for those samples for approximately 24 hours. This PREVI Isola® adapted streaking procedure with sputum liquefying protocol is a good standardized

Background

For clinical microbiology labs, a qualified specimen is in related to appropriate specimen collection and transportation. To efficiently diagnose the pathogens, the streaking technique which allows colonies sufficiently spread apart from each other is quite important for the following identification and susceptibility testing. Recently, clinical laboratory automation systems were developed to improve and accelerate the pathogen diagnosis work flow by standardizing the inoculation protocol. The automated inoculation system is acknowledged with cost-effective inoculation protocol with more number of isolated colonies, which allows lab staffs to read plates easier and faster.

Although automated inoculation system has been proven to receive consistent and more pure culture result. But not all samples are suitable for automation. Liquid Based specimen are suitable for automated plated streaker. For those non-liquid based specimens, liquid based transport media are needed. Or, the inoculation protocol needs to be modified. In our lab, sputum represents over 50% of the received samples. To make the automated system to be even more efficiently applied for our daily work flow, we evaluate a sputum liquefaction protocol along with the automated system.

Material and Method

Specimens:

Since 2015/08/05 till 2015/09/05 , we collected 185 clinical sputum as the study sample.

Manual Streaking Method:

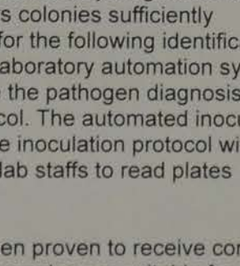
Received sputum were inoculated manually on to culture media using a three-quadrant method.

Sputum Liquefaction:

Prepare 5% N-acetyl-L-cysteine(NALC) with pH 7.4 PBS buffer. Fully mix same amount of sputum with 5%NALC solution by vortex for 15 seconds. Let the mixture standing for 30 minutes at room temperature. The liquefied samples are then applied for Previ Isola® plate streaking.

Automated Streaking Method:

PREVI® Isola (bioMérieux, Craponne, France) is an automated bacteriology lab system in processing of liquid microbiology specimen samples (i.e, urines, liquid transport media for swabs, and etc.) The system throughput is 180 plates/hour. 10µl of liquid specimen is inoculated onto media plate with instrument pippet, and then streaked with a disposable plastic comb in a circular motion.



Results and Discussion

After 24 hours incubation, all the plates in parallel were pictured and the variety of colonies, the quality of colony growth, and the possibility to isolate single colony from the two inoculation methodologies were investigated and compared (Figure 1).

Figure 1.
Agar plates inoculated with
(A)Previ Isola
(B)Manaul



Results and Discussion (Cont')

After the sputum liquefaction protocol, we found the performance of 183 out of 185 samples from manual and automated streaking methodology were the same. The protocol made sputum liquefied sufficiently. The colony grow quality, and the variety of colonies were the same between the two methodologies (Fugure 2). Due to the automated streaking methodology can maximize the agar surface inoculated, the liquefaction protocol with PREVI Isola revealed superior single colony isolation performance (Figure 3). Lab staffs might pick up single colonies from the automated streaking plates easier.

Figure 2

Same colony varieties can be found on agar plates inoculated with
(A)Previ Isola
(B)Manaul

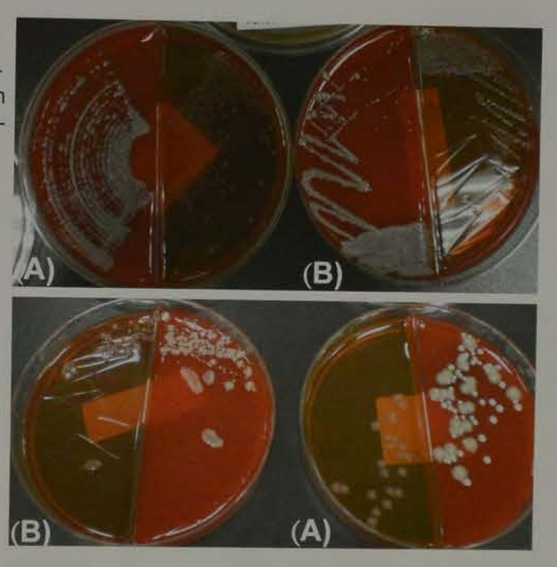
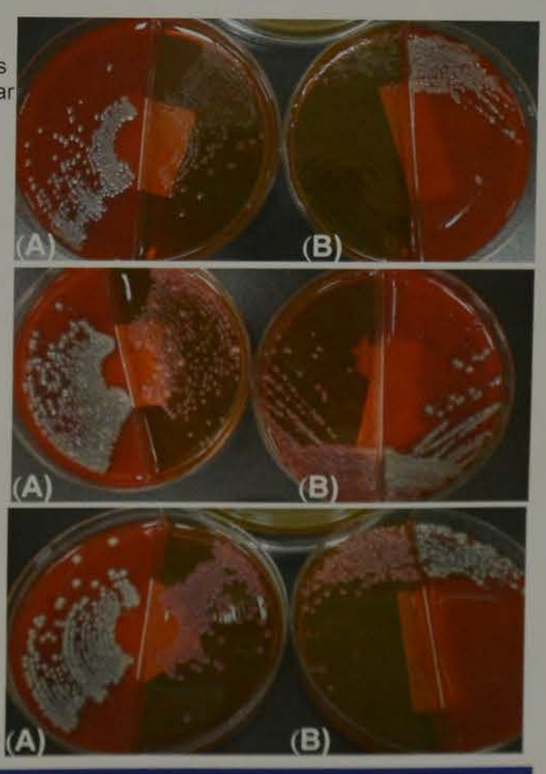


Figure 3

More single colonies can be found on agar plates inoculated with
(A)Previ Isola



Conclusion

The data illustrated that the modified inoculation protocol with mucolytic agents can help a lot for labs using automated system for viscosity specimen. Along with the advantages that the automated system enables the microbiology inoculation protocol to be more standardized and received more isolated colonies for subsequent identification and antimicrobial susceptibility testing, the modified protocol is helpful for clinical microbiology labs to improve efficiency in workflow via cost-saving with less plates and human resource, and time-saving for re-isolation.

Reference

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Microbiology

PA-37

PA-38

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The Role of Microbiology Laboratory in the Response to Formosa Fun Coast Dust Explosion

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Introduction

On June 27, 2015, a tragic starch-based powder explosion and fire accident on a music stage during a "Color Play Asia" party occurred at Formosa Fun Coast (Eight Immortals Paradise), which is a recreational water park in Bali District, New Taipei, Taiwan. According to the Taiwan Ministry of Health and Welfare, 15 fatalities were attributed to the. The explosion has been called the worst incident of mass injury in New Taipei.

Of the 508 young people who were injured, most of the patients experienced burns, and 221 people had severe burns and inhalation, placing them in critical condition. Because they were typically wearing only swimwear, most of the patients had large burn areas, with a mean total body surface area of 44%, reaching 80% in 22 victims. Most of the patients were tertiary students aged in their twenties and late teens, and they were treated in over 50 hospitals across Taiwan.

Among the 12 patients seeking medical treatment in our hospital, 10 received complete hospital courses(Table 1-2). Here, we describe microbiology lab experience.

Methods

Sample preparation for MALDI-TOF MS.

Fresh colony material was smeared on a polished steel MSP 96 target (Bruker Daltonik) using a toothpick. For the direct transfer-formic acid method, 1 µl of 70% formic acid was added to the bacterial spot and allowed to air. Then, overlaid with 1 µl of a saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonik), and air dried at room temperature.

MALDI-TOF MS analysis.

The acquisition and analysis of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonik) using the MALDI Biotyper software package. The Bruker bacterial test standard (Bruker Daltonik) was used for calibration according to the instructions of the manufacturer.

Phoenix system antimicrobial susceptibility testing.

The assay is a broth-based microdilution test. The system uses a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. AST broth was supplemented with one drop of indicator. A 25-ml aliquot of ID suspension (0.5 – 0.6 McFarland) was transferred to AST broth, and the AST broth was then poured into the Phoenix NMIC-64 & PMIC-65 panel.

Results

Table 1. Length of Stay is displayed against Burn Depth

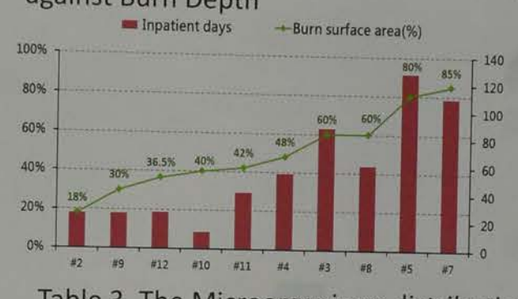


Table 2.Burn patients specimen types

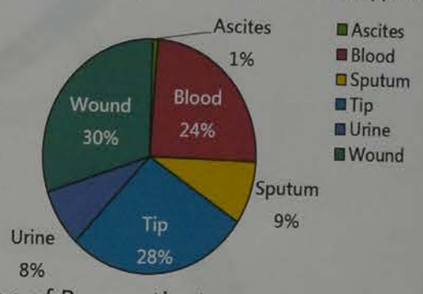


Table 3. The Microorganisms distribution of Burn patients.

	Microorangnism was isolated	<2 Weeks	2-4 Weeks	4-6 Weeks	6-8 Weeks	8-10 Weeks	10-12 Weeks	>12 Weeks	Total
GNF	*Acinetobacter baumannii*	4	2	3	0	1	1	0	11
	Pseudomonas aeruginosa	2	0	0	1	0	0	0	3
	Chryseobacterium meningosepticum	3	0	0	0	0	0	0	3
	Stenotrophomonas maltophilia	0	1	2	0	0	0	0	3
	Chryseobacterium indologenes	1	0	0	0	0	0	0	1
	Sphingomonas paucimobilis	1	0	0	0	0	0	0	1
GNB	*Klebsiella pneumoniae*	0	7	2	0	1	0	0	10
	Escherichia coli	0	0	1	0	1	0	0	2
	Proteus mirabilis	0	0	0	3	0	0	0	3
	Providencia stuartii	0	0	0	2	0	0	0	2
	Aeromonas hydrophila	1	0	0	0	0	0	0	1
GPC in cluster	*Staphylococcus aureus*	1	4	5	1	1	0	1	13
	Staphylococcus epidermidis	0	2	3	0	0	0	0	5
	Staphylococcus haemolyticus	0	3	1	0	0	0	0	4
	Staphylococcus cohnii	0	0	1	0	0	0	0	1
	Staphylococcus hominis	0	0	1	0	0	0	0	1
	Staphylococcus saprophyticus	0	0	1	0	0	0	0	1
GPC in chain	*E. faecalis*	5	0	1	0	0	0	0	6
	E. faecium	3	0	0	0	0	0	0	3
	Strepto. uberis	1	0	0	0	0	0	0	1
	Streptococcus mitis	1	0	0	0	0	0	0	1
Yeast-like	*Yeast-like*	2	7	1	0	0	0	5	15
	Candida tropicalis	0	1	0	0	0	0	1	2
	Aspergillus fumigatus	0	0	1	0	0	0	0	1

Discussion

The experience of taking care of multiple massive deep burn-injury patients generates some conclusions:

- Cooling burn-injury by dirty swimming pool water may facilitate environmental bacteria causing infections.
- Bacteria translocation from intestine and defected skin may eventually cause bacteremia and septic shock.
- Medical staff may cause bacteria inter-patient outbreak during daily care and procedures.
- Construction works should keep away from these immunocompromised patients to avoid fungal, such as Aspergillosis, infections. Strict infection-control procedures should be performed to protect these massive burn-injury patients.

Microbiology laboratory professionals may take efforts to collect samples from accidental scene as first-line database for burn-injury wound contaminations. Rapid test for bacteria species, such as MALDI-TOF, should be used for early detection and early intervention. Considering the feature of burn-injury wounds, every wound culture should be isolated and cultures by detail, not only the predominate pathogens. The species analyses and susceptibility test should be performed on every yeast samples(Table 3). By the cooperation between laboratory experts and clinical doctors, better outcome of the massive burn-injury victims can be expected.

Microbiology

PA-40



Evaluation of the Chromogenic Agar chromID C. difficile

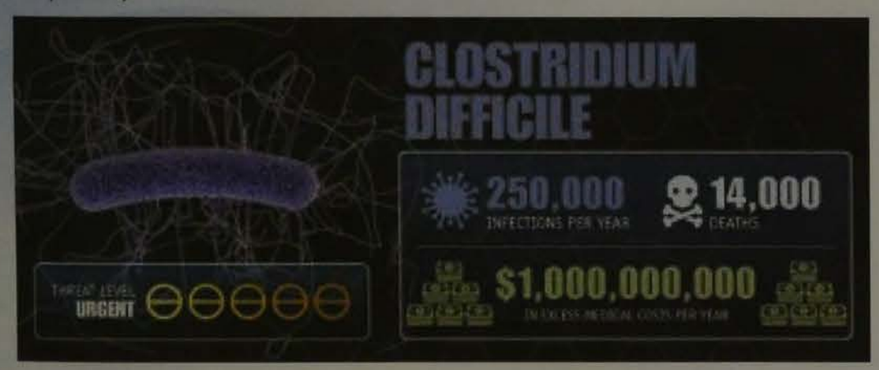
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Introduction

Clostridium difficile is a Gram-positive, spore-forming bacterium that is an obligate anaerobe and a potentially fatal gastrointestinal pathogen of humans and animals. Initially described in 1935 as a commensal organism found in fecal samples from newborns, C. difficile was later demonstrated to be the causative agent of pseudomembranous colitis associated with antibiotic treatment. C. difficile infections (CDI) are typically preceded by antibiotic treatment which results in the disruption of the normal colonic flora, creating a niche for C. difficile to thrive. C. difficile is transmitted as a dormant spore via the fecal-oral route and subsequently germinates within the gastrointestinal tract, producing vegetative cells capable of generating several toxins and causing severe disease and colitis. CDI are often refractory to conventional treatments and these infections are frequently recurrent.



C. difficile is very sensitive to even low levels of oxygen in the environment. For C. difficile to persist in the environment and be efficiently transmitted from host to host, the formation of a metabolically inactive spore is critical. Because the laboratory maintenance and manipulation of C. difficile requires a controlled, anaerobic environment, these techniques necessitate the use of an anaerobic chamber. Use of anaerobic chambers has resulted in increased recovery and isolation of obligate anaerobes, and has allowed a number of molecular techniques to be performed in an anaerobic atmosphere.



Toxigenic culture is the new reference standard for the diagnosis of C. difficile infection (CDI). This two-step method consists of isolating C. difficile strains on a selective medium after 48 h of incubation in an anaerobic atmosphere and then testing the colonies for their potential to produce toxins. This method is time consuming, but in addition to its diagnostic purpose, culture is essential for strain typing and antimicrobial susceptibility testing. Many selective media containing antibiotics such as cycloserine or cefoxitin are ommercially available. They are usually derived from the CCFA medium (egg yolk-fructose base with cycloserine and cefoxitin) described by George et al. in 1979 . Some media also contain taurocholate or lysozyme to enhance spore germination and improve sensitivity or p-hydroxyphenylacetic acid for presumptive identification of C. difficile . Despite the use of antibiotics, the selectivity of the media is relatively poor, and ethanol shock and heat shock have been proposed as methods for selecting spores and optimizing the recovery of C. difficile strains both from clinical samples and from the environment . The main advantage of these techniques is to limit the growth of contaminating endogenous flora and to facilitate the reading of the plates.

Material and method

The objective of this study was to evaluate the performance of the chromID C. difficile agar (bioMérieux) and chromID C. difficile agar (C.M.P), a new chromogenic medium for the isolation of C. difficile in 24 h, compared to another selective media:CCFA.



In this study , we collected 32 samples that consecutive diarrheal stools from patients suspected of having CDI were included(GDH result positive). Culture was performed within 24 h to 72 h of collection, and stools were stored at -20°C until processing.





Presumptive identification was based on colony aspects (black colonies on the chromID C. difficile agar or Fluorescent present), odor, Gram staining, and results obtained with the PCR data.

primer	sequence
16s RNA forward	5' GCTGGATCACCTCCTTTCTAAG
23s RNA reverse	5' TGACCAGTTAAAAAGGTTTGATAGATT

Result

1. In this study , we found 32 samples were culture positive for C.difficile in both selective agar.
2. In chromID C. difficile agar (bioMérieux) , the C.difficile colony is black , and after 48 hours , all of the bacteria growth.
3. In chromID C. difficile agar (C.M.P), the C.difficile colony is blue(under 365 nm) and after 72 hours , all of the bacteria growth.





Summary

Culture allows isolation of C. difficile strains, but a second step is necessary to determine the in vitro toxigenicity of the strain. The use of a chromogenic medium that was recently evaluated by Darkoh et al., which contains a chromogenic substrate that differentiates toxigenic from nontoxigenic strains in a single step, could be a good optionIdentification of C. difficile on chromID C. difficile agar in 24 h is easy due to the color of the colonies and its selectivity. However,this medium exhibits the best sensitivity at 48 h.

Reference

1. George WL, Sutter VL, Citron D, Finegold SM. 1979. Selective and differential medium for isolation of Clostridium difficile. J. Clin. Microbiol. 9:214–219.
2. Foster NF, Riley TV. 2012. Improved recovery of Clostridium difficile spores with the incorporation of synthetic taurocholate in cycloserinecefoxitin-fructose agar (CCFA). Pathology 44:354–356.
3. Wilcox MH, Fawley WN, Parnell P. 2000. Value of lysozyme agar incorporation and alkaline thioglycollate exposure for the environmental recovery of Clostridium difficile. J. Hosp. Infect. 44:65–69.

Microbiology

PA-41

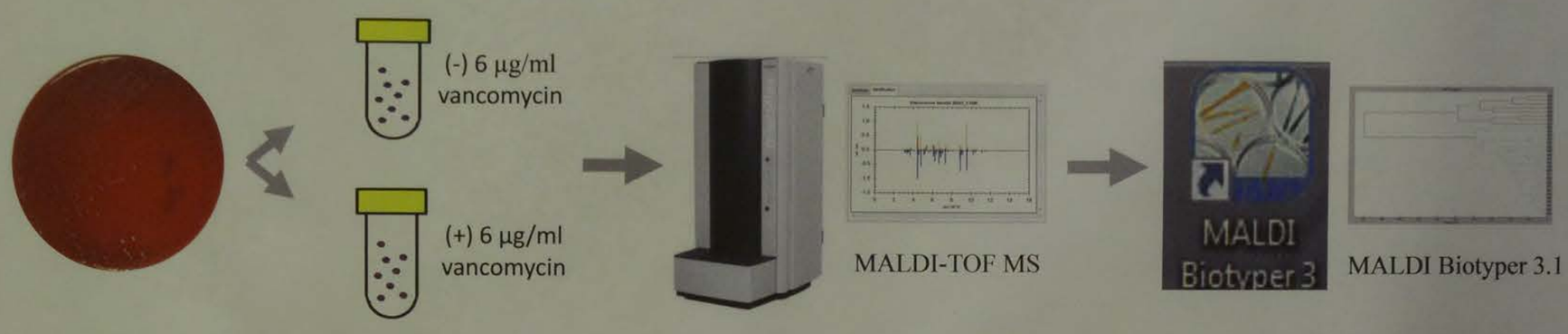
Application of MALDI-TOF MS and MALDI Biotyper for Analyzing the Susceptibility of *Enterococcus* to Vancomycin

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PA-41

Backgrounds

- The infection of Vancomycin-resistant *Enterococci* (VRE) is higher than each year in the world.
- Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been applied as a rapid, accurate, and inexpensive method for bacterial identification in clinical laboratories.



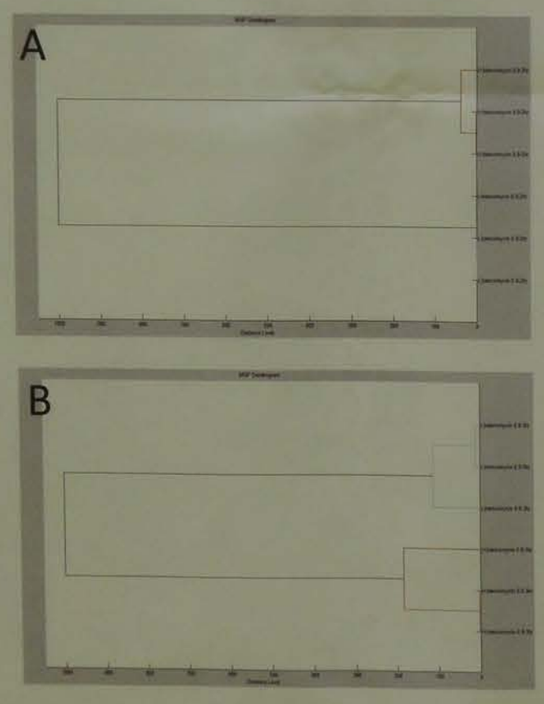


Fig1. The distance level of dendrograms were higher in the bacterial density of $6x10^8$ CFU/ml and the incubation time of 2-3 hours than those in the other bacterial density and the other incubation time.(A) bacterial density of $6x10^8$ CFU/ml and the incubation time of 2 hours ; (B) bacterial density of $6x10^8$ CFU/ml and the incubation time of 3 hours.

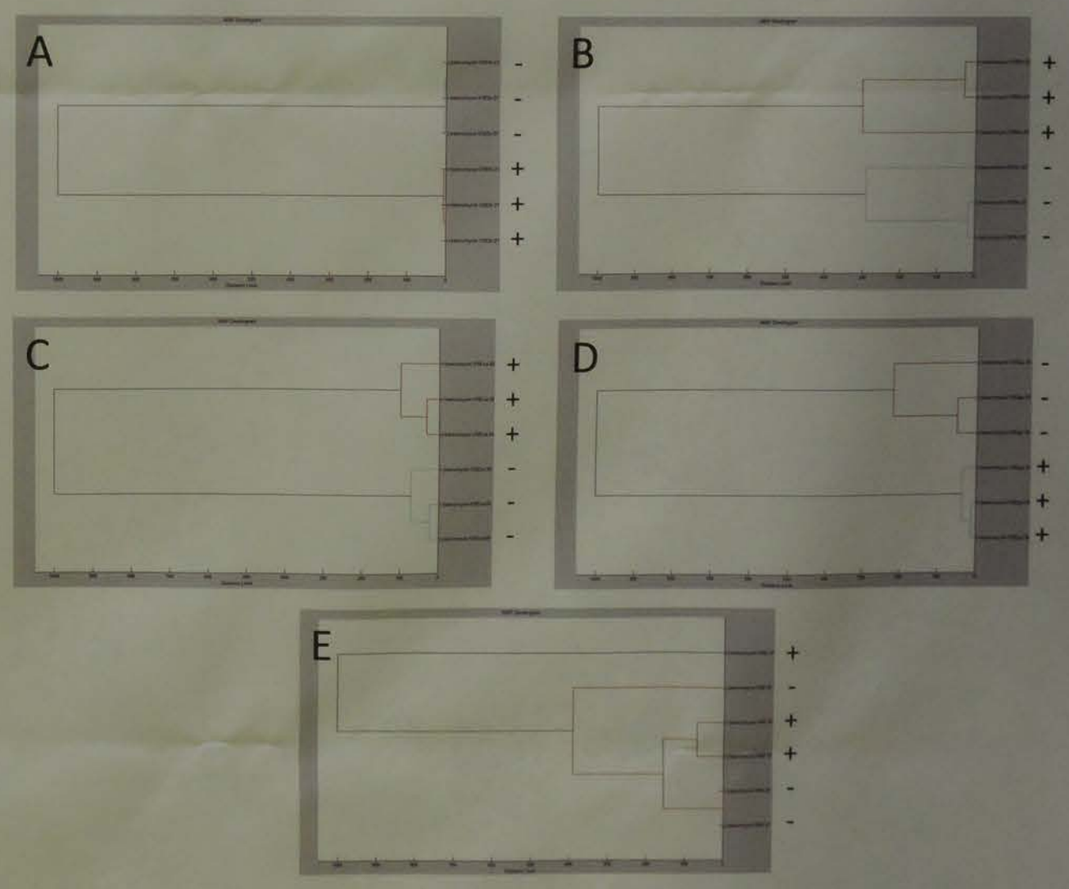


Fig2. The discrimination of VRE and Vancomycin-susceptible *Enterococci* (VSE).
(A) *E. faecalis* ; (B) *E. faecium* ; (C) *E.casseliflavus* ; (D) *E. gallinarum* ; (E) *E. faecium* (VRE).

Summary

- The results indicated that the method was able to discriminate the VRE and VSE in different group.
- The combination of MALDI-TOF MS and MALDI Biotyper may be applied as a potential tool for rapid detection of Vancomycin-resistant *Enterococci*.

Microbiology

PA-42

Effect of cultured metabolites on the pel genes expression of *Pseudomonas aeruginosa*

PA-42

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Backgrounds :

- The exopolysaccharides existed in *P. aeruginosa* biofilm include alginate, Psl, and Pel.
- The processes for biofilm formation include adherence, proliferation, and dispersion phases.
- Both adhesive factors and disruptive factors are important in biofilm formation.
- The genes associated with Pel formation are located in *pel* operon (*pel*A-G).



Structure of pel gene cluster



Fig.1. The pellicle formation of *Pseudomonas aeruginosa* (1-5 day).



Fig.2. The Congo red stained exopolysaccharide of *Pseudomonas aeruginosa* (1-5 day).

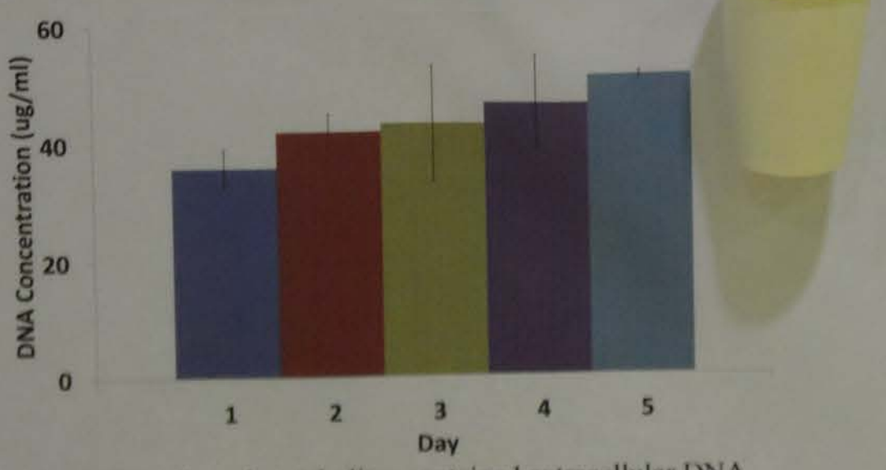


Fig.3. The cultured metabolites contained extracellular DNA.



Fig.4. The cultured metabolites contained protein.



Fig.5. The cultured metabolites contained pyocyanin.

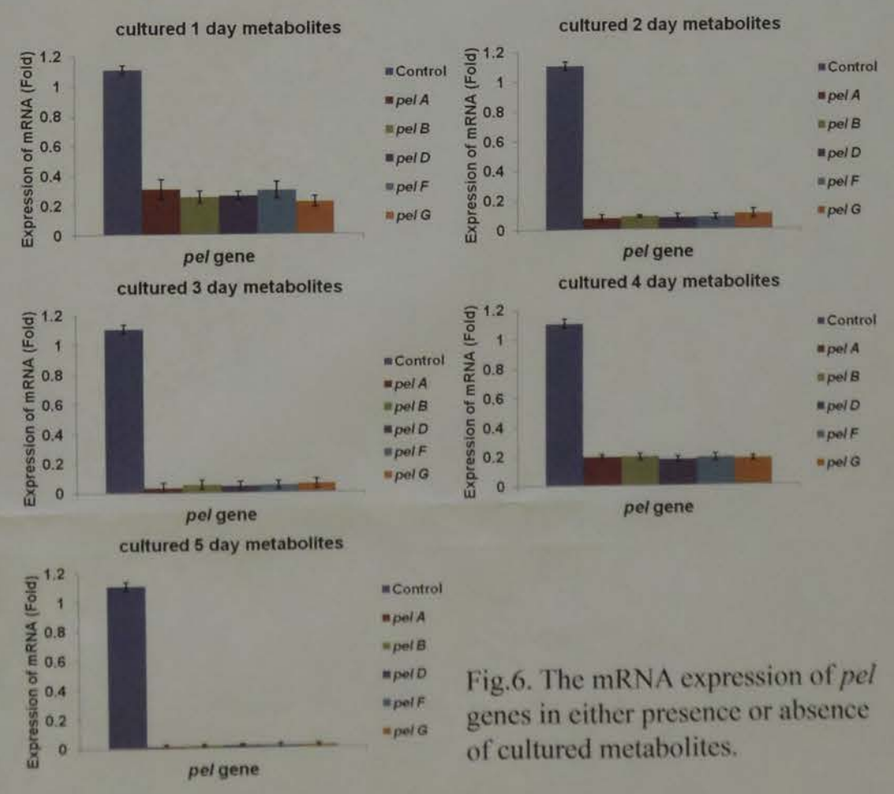


Fig.6. The mRNA expression of *pel* genes in either presence or absence of cultured metabolites.

Conclusions :

1. The cultured metabolites contained extracellular DNA, protein and pyocyanin.
2. The mRNA expressions of pel genes were shown to be decreased in medium by adding the cultured metabolites.

Microbiology

PA-43

Alanine uptake effects on methicillin-resistant *Staphylococcus aureus* cell surface charge and sensitivity to nisin

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Abstract

Background: The drug sensitivity of methicillin-resistant *S. aureus* (MRSA) to current antibiotics was found decreasing in these years. Looking for an alternative antibacterial agent has become urgent. ***Objective:*** The goal of this study is to analyze the antibacterial effects of nisin against various *S. aureus* strains, and, at the same time, to evaluate the effect of alanine uptake on the change in cell surface charges, following which their drug sensitivity may increase. ***Material and Method:*** In this study thirty one MRSA strains and five methicillin-sensitive *S. aureus* (MSSA) strains, isolated clinically, were utilized for assaying their sensitivities to nisin after uptake of L-alanine. ***Result:*** Results demonstrated that the minimum inhibitory concentrations (MICs) of vancomycin for these MRSA strains were ranged between 0.5~1.0μg mL^{-1}, and the MICs of nisin for these MRSA strains were ranged between 1.25 – 20 IU mL^{-1} with an average of 10 IU mL^{-1}. Similar MIC range, 5-20 IU mL^{-1} , with an average of 10 IU mL^{-1}, was obtained for MSSA stains. An increased sensitivity of most of the MRSA (77%) to nisin was found, with average MIC_{nisin} reduced to 49 %, after being cultured with L-alanine supplement (1%, w/v); it seemed that MRSA with MIC_{Va} of 1.0μg mL^{-1} was affected most significant. The cytochrome *c* (cyt C) unbound test results demonstrated the L-alanine uptake could decrease the bounding capacity of cytC to strains, which indicated that the negative charge on cell surface was decrease. ***Conclusion:*** The study found that the MRSA strain with less negative charge on cell surface was more vulnerable to nisin. These findings will be helpful in investigating for alternative antibacterial agents against MRSA.

Meterials and methods

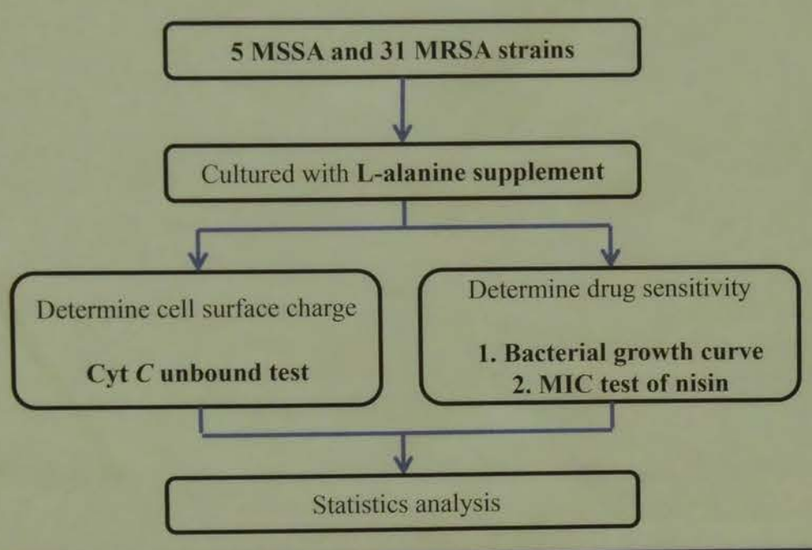


Table 1. The influence on MICnisin of MRSA after 1% of L-alanine treatment

strains	MICnisin (IU/mL)		Va MIC (μg/ml)	Ala^-/Ala^+	strains	MICnisin (IU/mL)		Va MIC (μg/ml)	Ala^-/Ala^+
	Ala^-	Ala^+				Ala^-	Ala^+		
MSSA 1	5	5	≦0.5	1	MRSA 14	10	≦5	≦0.5	≧2
MSSA 2	5	10	≦0.5	0.5	MRSA 15	10	≦2.5	≦0.5	≧4
MSSA 3	10	10	≦0.5	1	MRSA 16	10	5	1	2
MSSA 4	10	10	≦0.5	1	MRSA 17	10	5	1	2
MSSA 5	20	10	1	2	MRSA 18	10	≦2.5	1	≧4
MRSA 1	2.5	≦1.25	1	≧2	MRSA 19	10	5	1	2
MRSA 2	2.5	≦1.25	1	≧2	MRSA 20	10	≦2.5	1	≧4
MRSA 3	2.5	≦1.25	1	≧2	MRSA 21	10	1.25	1	8
MRSA 4	5	≦2.5	≦0.5	≧2	MRSA 22	20	10	≦0.5	2
MRSA 5	5	≦2.5	1	≧2	MRSA 23	20	≦5	1	≧4
MRSA 6	5	2.5	1	2	MRSA 24	20	5	1	4
MRSA 7	5	≦1.25	1	≧4	MRSA 25	2.5	2.5	1	1
MRSA 8	10	≦5	1	≧2	MRSA 26	5	5	≦0.5	1
MRSA 9	10	≦5	1	≧2	MRSA 27	5	5	1	1
MRSA 10	10	5	≦0.5	2	MRSA 28	10	10	≦0.5	1
MRSA 11	10	≦5	1	≧2	MRSA 29	10	10	1	1
MRSA 12	10	≦5	1	≧2	MRSA 30	10	10	≦0.5	1
MRSA 13	10	≦5	1	≧2	MRSA 31	10	10	≦0.5	1

Results

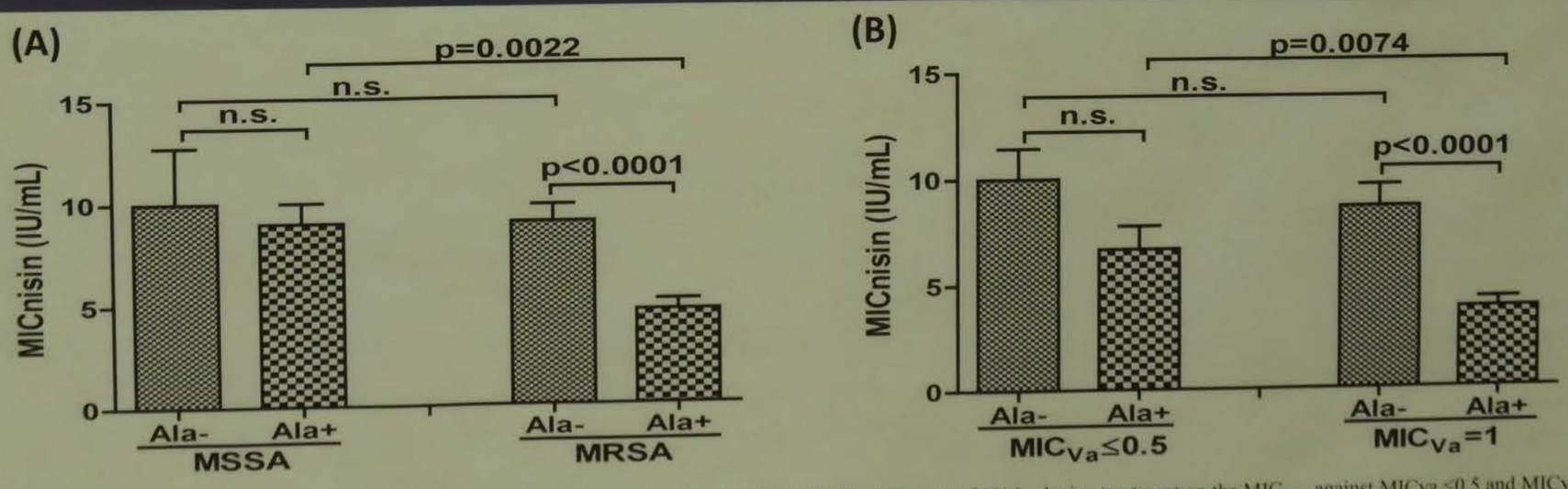


Fig. 1. The influence of 1% L-alanine treatment on the MIC_{nisin} against MSSA and MRSA**(A).** The influence of 1% L-alanine treatment on the MIC_{nisin} against MICva ≤0.5 and MICva= 1.0 μg/ml of MRSA**(B). The** 1% L-alanine treatment was significant reduced the MIC_{nisin} in MRSA. The MICva= 1.0 μg/ml of MRSA has higher susceptibility to nisin in MRSA.

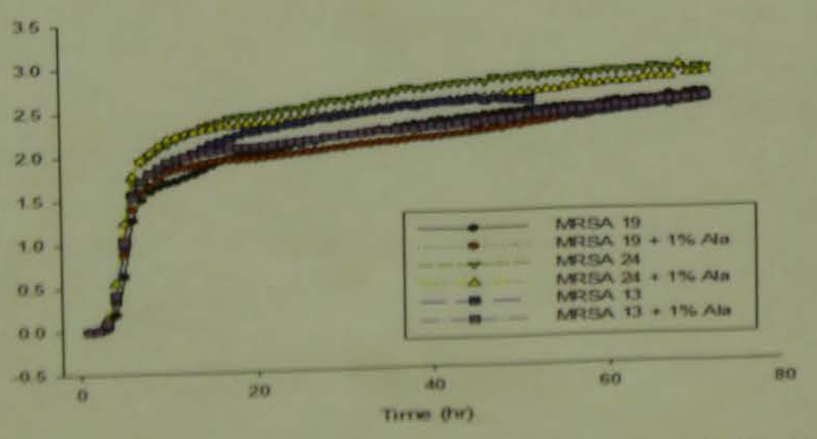


Fig. 2. The influence of L-alanine treatment on the growth curves of MRSA stains (No. 14 - 17 and 20). The 1% L-alanine treatment had no significant effect on MRSA strains growth.

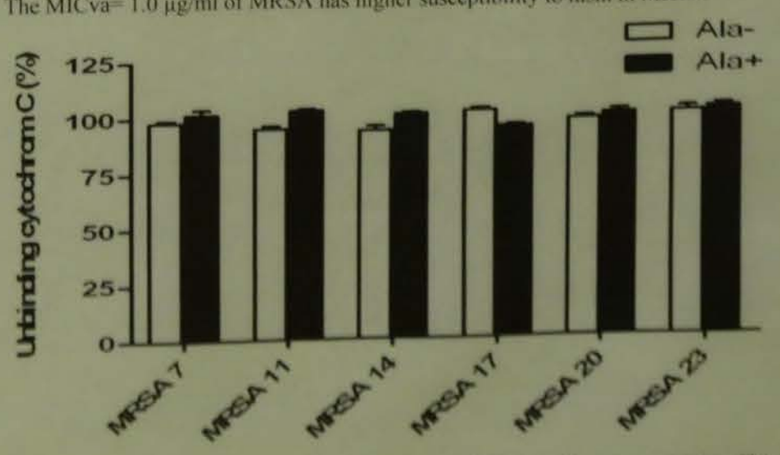


Fig. 3. The influence of L-alanine treatment on MRSA cell surface charges. The 1% L-alanine treatment had slightly increased the unbinding cytochrom *c* percentage of MRSA strain.

Conclusion and Discussion

1. Nisin has similar antibacterial effects on MRSA and MSSA. However, after incubated with 1% L-alanine, the nisin susceptibility of MRSA is stronger than MSSA, especially the MRSA strains with MICva of 1.0 (μg/ml)
2. The surface charge of MRSA, according to cytochrome *c* test, can be **decreased** after 1% L-alanine treatment.
3. Our data suggest that nisin has better antimicrobial activity against MRSA after L-alanine treatment , especially the strains more resistant to vancomycin; and the sensitivity of MRSA strains to nisin may be associated with their surface charges.

Acknowledgements

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Microbiology

PA-44

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CHHW

Evaluation of Drug Susceptibility Testing with the Broth Microdilution Method in Multidrug-resistant Mycobacterium tuberculosis

The accuracy and turn-around-time of Drug Susceptibility Testing in Multidrug-resistant Mycobacterium tuberculosis

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Purpose

Multidrug-resistant (MDR) Mycobacterium tuberculosis (TB) has increased in recent years. Drug susceptibility testing (DST) must be rapidly concluded for effective treatment the disease. Agar proportion method (APM) is the gold standard assay of DST in TB, but it lacks of precision in some drugs and MICs data to support chemotherapy. A commercial MICs susceptibility plates for TB using broth microdilution method (BMM), MYCOTBI (TREK, England) was imported in Taiwan recently. The aim of this study was to compare MYCOTBI and APM for DST in clinical MDRTB isolates, and combined with detection of drug resistant genes, if necessary.

Methods

A total of 97 clinical MDRTB isolates were included and using H37Rv strain as control. Twenty-seven isolates were analyzed in the preliminary test to confirm the ability of operation staff. Two of the other sixty isolates were excluded because of no enough control colonies grown. MYCOTBI was performed in 96-well microtitre plates containing 12 drugs at appropriate dilutions. APM was performed as described in CLSI M24-A2. The results of resistant and susceptible were defined as positive and negative, respectively.

Results

The results of preliminary test were showed in table 1 and table 2, and the secondary stage study were showed in table 3 ~ table 9. Results could be read about 18 days after inoculation in BMM and 21 days of APM. The consistent rate of BMM and APM in the first line drugs, Soniazid, Rifampicin, Ethambutol, Streptomycin and the second line drugs, Ethionamide, Kanamycin, Ofloxacin, Para-aminosalicylic acid were 96.6%(56/58), 100%(58/58), 86.2%(50/58), 91.4%(53/58), 94.8%(55/58), 96.6%(56/58), 98.3%(57/58) and 96.6%(56/58), respectively. Reevaluation the performance at BMM with adding the results of detection of drug resistant genes in some inconsistent isolates. The new consistent rates of Soniazid, Rifampicin, Ethambutol, Kanamycin and Ofloxacin were 98.3%(57/58), 100%(58/58), 92.5%(55/58), 100%(58/58) and 100%(58/58), respectively.

Table 1. Comparison the 1st line drugs in the preliminary study

Drug	APM results	BMM results: No. of resistant strains	No. of susceptible strains	% sensitivity	% specificity
INH	Resistant	21	3	87.5%	100.0%
	Susceptible	0	3		
RIF	Resistant	20	2	90.9%	80.0%
	Susceptible	1	4		
STR	Resistant	13	3	81.3%	90.9%
	Susceptible	1	10		
EMB	Resistant	6	7	46.2%	92.9%
	Susceptible	1	13		

APM: Agar proportion method ; BMM: Broth Microdilution Method

Table 2. Comparison of the 2nd line drugs in the preliminary study

Drug	APM results	BMM results: No. of resistant strains	No. of susceptible strains	% sensitivity	% specificity
EA	Resistant	0	4	0.0%	100.0%
	Susceptible	0	5		
KM	Resistant	1	0	100.0%	100.0%
	Susceptible	0	8		
OFX	Resistant	2	0	100.0%	85.7%
	Susceptible	1	6		
PAS	Resistant	2	3	40.0%	100.0%
	Susceptible	0	4		

Table 3. The inconsistent rate within different staffs of APM

	Resistant	Susceptible	Inconsistent	Resistant%	Inconsistent%
INH	55	3	0	94.8%	0.0%
RIF	55	3	1	94.8%	1.7%
EMB	40	18	3	69.0%	5.2%
STR	36	22	3	62.1%	5.2%
EA	23	35	4	39.7%	6.9%
KM	16	32	0	27.6%	0.0%
OFL	9	49	2	15.5%	3.4%
PAS	7	51	1	12.1%	1.7%

Table 4. The inconsistent rate within different staffs of BMM

	Resistant	Susceptible	Inconsistent	Resistant%	Inconsistent%
INH	55	3	1	94.8%	1.7%
RIF	55	3	4	94.8%	6.9%
EMB	32	26	4	55.2%	6.9%
STR	31	27	7	53.4%	12.1%
EA	23	35	5	39.7%	8.6%
KM	16	42	0	27.6%	0.0%
OFL	8	50	6	13.8%	10.3%
PAS	9	49	6	15.5%	10.3%

Table 5. Comparison of BMM and APM in the 1st line drugs

Drug	APM result	BMM result: Resistant	Susceptible	Consistent	Sensitivity	Specificity	PPV	NPV
INH	Resistant	54	1	96.6%	98.2%	66.7%	98.2%	66.7%
	Susceptible	1	2					
RIF	Resistant	55	0	100.0%	100.0%	100.0%	100.0%	100.0%
	Susceptible	0	3					
EMB	Resistant	32	8	86.2%	80.0%	100.0%	100.0%	69.2%
	Susceptible	0	18					
STR	Resistant	31	5	91.4%	86.1%	100.0%	100.0%	81.5%
	Susceptible	0	22					

Table 6. Comparison of BMM and APM in the 2nd line drugs

Drug	APM result	BMM result: Resistant	Susceptible	Consistent	Sensitivity	Specificity	PPV	NPV
EA	Resistant	22	2	94.8%	91.7%	97.1%	95.7%	94.3%
	Susceptible	1	33					
KM	Resistant	15	1	96.6%	93.8%	97.6%	93.8%	97.6%
	Susceptible	1	41					
OFL	Resistant	8	1	98.3%	88.9%	100.0%	100.0%	98.0%
	Susceptible	0	49					
PAS	Resistant	7	0	96.6%	100.0%	96.1%	77.8%	100.0%
	Susceptible	2	49					

Table 7. Comparison of APM and drug resistant gene detection

Drug	genotyping result	APM result: Resistant	Susceptible	Consistent	Sensitivity	Specificity	PPV	NPV
INH	Resistant	50	0	91.4%	100.0%	37.5%	90.9%	100.0%
	Susceptible	5	3					
RIF	Resistant	54	2	94.8%	96.4%	50.0%	98.2%	33.3%
	Susceptible	1	1					
EMB	Resistant	27	6	67.2%	81.8%	48.0%	67.5%	66.7%
	Susceptible	13	12					
KM	Resistant	13	0	94.8%	100.0%	93.3%	81.3%	100.0%
	Susceptible	3	42					
OFL	Resistant	7	0	96.6%	100.0%	96.1%	77.8%	100.0%
	Susceptible	1	49					

Table 8. Comparison of BMM and drug resistant gene detection

Drug	genotyping result	BMM result: Resistant	Susceptible	Consistent	Sensitivity	Specificity	PPV	NPV
INH	Resistant	50	0	91.4%	100.0%	37.5%	90.9%	100.0%
	Susceptible	5	3					
RIF	Resistant	54	2	94.8%	96.4%	50.0%	98.2%	33.3%
	Susceptible	1	1					
EMB	Resistant	24	9	70.7%	72.7%	68.0%	75.0%	65.4%
	Susceptible	8	17					
KM	Resistant	13	0	94.8%	100.0%	93.3%	81.3%	100.0%
	Susceptible	3	42					
OFL	Resistant	7	0	98.3%	100.0%	98.0%	87.5%	100.0%
	Susceptible	1	50					

Table 9. Comparison of BMM and APM modified with drug resistant gene detection

Drug	APM result	BMM modified by genotyping: Resistant	Susceptible	Consistent	Sensitivity	Specificity	PPV	NPV
INH	Resistant	55	0	98.3%	100.0%	66.7%	98.2%	100.0%
	Susceptible	1	2					
RIF	Resistant	55	0	100.0%	100.0%	100.0%	100.0%	100.0%
	Susceptible	0	3					
EMB	Resistant	27	3	94.8%	82.5%	100.0%	100.0%	85.7%
	Susceptible	0	18					
KM	Resistant	16	0	100.0%	100.0%	100.0%	100.0%	100.0%
	Susceptible	0	42					
OFL	Resistant	9	0	100.0%	100.0%	100.0%	100.0%	100.0%
	Susceptible	0	49					

Conclusion

MYCOTBI had the advantages to shorten turn-around-time of DST and gave MICs information. The concordance of BMM and APM was similar with previous literatures, even most of the study materials were drug-resistant isolates in this study but susceptible in previous literatures. The performance of BMM was superior when adding the results in detection of drug resistant genes. So BMM had the potential to replace APM in clinical laboratories.

Microbiology

PA-45

Investigate Clinical Laboratory signs of patients with Mycoplasma pneumoniae community -acquired pneumonia in Pingtung Taiwan

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Background & Aim

Mycoplasma pneumoniae (MP) is one of the main causes of atypical community-acquired pneumonia (CAP). This retrospective study is aimed to investigate the clinical and laboratory profile in patients with suspected MP-associated pneumonia.

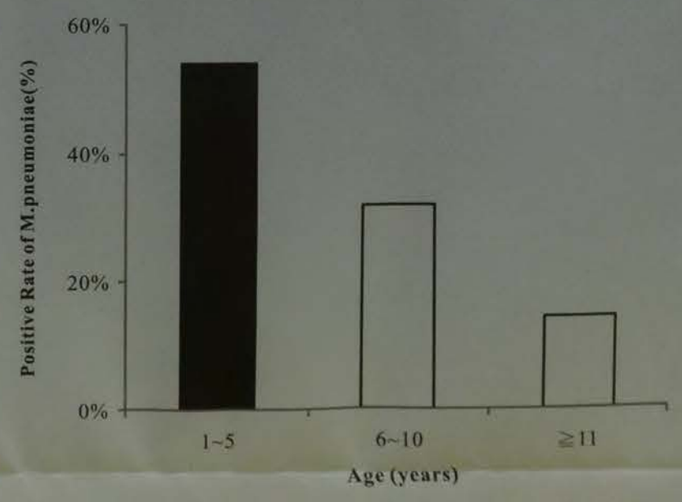
Materials and Methods

186 patients with clinical symptoms suggestive of pneumonia during the period from October to December 2015 showed serum MP-specific IgM antibody positive. Exclusion criteria included patients with gastroenteritis, influenza (cough, fever, rale), and chronic respiratory disease (asthma, pulmonary tumor). The diagnosis of MP infection was based on serological testing of antibodies by two MP-IgM ELISA systems in Enzy-Well (Italy) or ImmunoWELL (USA). The patients were divided into 2 groups based on IgM analysis: MP negative pneumonia and MP positive pneumonia. The cutoff criteria includes: an index value were defined as optical density (OD) sample value/OD cutoff value >1.1 (Enzy-Well) and serum MP -IgM levels>770 U/ml (ImmunoWELL).

Table 1. Baseline demographic details of patients with CAP caused by MP

Characteristics	MP pneumonia (n=63)	%
Males	35	56%
Females	28	44%
Age group (years)	6.38±3.99	
1~5	34	54%
6~10	20	32%
≧11	9	14%
total	63	100%

Figure 1. Age-specific rates of infection in patients with CAP caused by MP



Results

63 of 186 (34%) patients with suspected pneumonia showed serum MP-specific IgM antibody positive. The gender distribution of MP-infected patients was 35 (56%) males and 28 (44%) females (Tab.1) . The mean ± standard deviation (SD) age of MP positive pneumonia was 6.38 ± 3.99 years old. The patient aged from 1 to 5 years had the highest positive rate of MP and ≧11 years with the lowest positive rate of MP (Fig.1, 54% versus 14%). There were no significant differences in laboratory profile (Tab.2,AST, ALT, white blood cell, neutrophil, lymphocyte, platelets, and sodium, all p-values >0.05) between these groups. Non-MP group had more patients with high CRP levels than MP group, but the percentage of CRP and ALT are not consistent with other study (Tab.2, underline).

Table 2. Normal range and pathological laboratory findings in patients with CAP caused by MP and non-MP

Laboratory finding	Normal range	Pathological finding: MP pneumonia	non-MP pneumonia	ref. MP pneumonia[a]	p-value* (MP versus non-MP)
CRP↑	10 mg/l	2/58 (3%)	14/112 (13%)	86/95 (91%)	0.044
AST↑	11–38 IU/l	7/33 (21%)	19/64 (30%)	36/147 (24%)	0.260
ALT↑	12–48 IU/l	0/34 (0%)	2/59 (3%)	29/ 147 (20%)	0.400
WBC↑	4–10*10³/ul	33/60 (55%)	60/113 (53%)	61/147 (41%)	0.469
Neutrophil↑	39–74 %	16/60 (27%)	26/113 (23%)	n.a.	0.361
Lymphocyte↑	19–48 %	6/60 (10%)	17/113 (15%)	n.a.	0.247
Platelets↓	150–400 *10³/ul	2/60 (3%)	5/113 (4%)	n.a.	0.539
Sodium↓	138–146 mmol/l	13/16 (81%)	35/42 (83%)	74/147 50%	0.564

↑, Increased values ;↓, decreased values; n.a., not appliable.
CRP, C-reactive protein ; WBC, white blood cell ;
AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Statistical analysis : Fisher's exact one-tailed test.
[a]Puljiz, I., et al., Epidemiol Infect, 2006. 134(3): p. 548-55.

Conclusions

34% of suspected pneumonia during the study period showed MP positive in Pingtung Taiwan. Most MP positive pneumonia was occurred in the 1-5 years age group. There were no significant differences in laboratory profile between MP positive and negative pneumonia. Since the detection of MP antibodies have limited sensitivity,PCR could be considered as an alternative for diagnosis of MP.

PA-46

Evaluating the quality of specimen before sputum culture to reduce inappropriate antibiotics prescription

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[2]Department of Laboratory Medicine, Taipei Medical University Hospital Taipei, Taiwan.

Introduction

The result of sputum culture is the gold standard of antibiotic treatment for pneumonia. If sputum specimen is poor quality, such as contaminated with oropharyngeal colonizers, it may result in inappropriate antibiotic usage, prolonged hospitalization, and increased antibiotic resistance. In addition, the contaminated specimen also increase the overload of laboratory staff and medical expenditure. In order to improve the quality of sputum specimen, we introduced the Murray and Washington guideline (Table 1), and according to the procedure of sputum culture announced by Taiwan Society of Laboratory Medicine. We spread the guideline to all medical practitioners, including the physicians and nurses. The guideline indicated that if the epithelial cell <25 and neutrophil>25 at low-power field (LPF), the specimen is good quality. Then, we perform this sputum culture can obtain the true pathogens.

Methods

From March 2014, each sputum culture specimen was accompanied with an order of Gram stain in our hospital. According to the guideline, we rejected the specimen for culture and report as mixed flora, then remark saliva contamination if the epithelial cell >25/LPF. We will not perform further identification of isolates and their antimicrobial susceptibility test. The positive rate of sputum culture in two stages (February2013-February 2014 and March 2014- March 2015) were analyzed by Z-test. The antibiotics prescription amount was presented by international scale as defined daily dose (DDD)/1000 patient day (PD). The difference of antibiotics prescription amount of these two stages was analyzed by Wilconxon Rank Sum Test.

Table 1. Murray and 's Grading System Quality of Sputum Samples

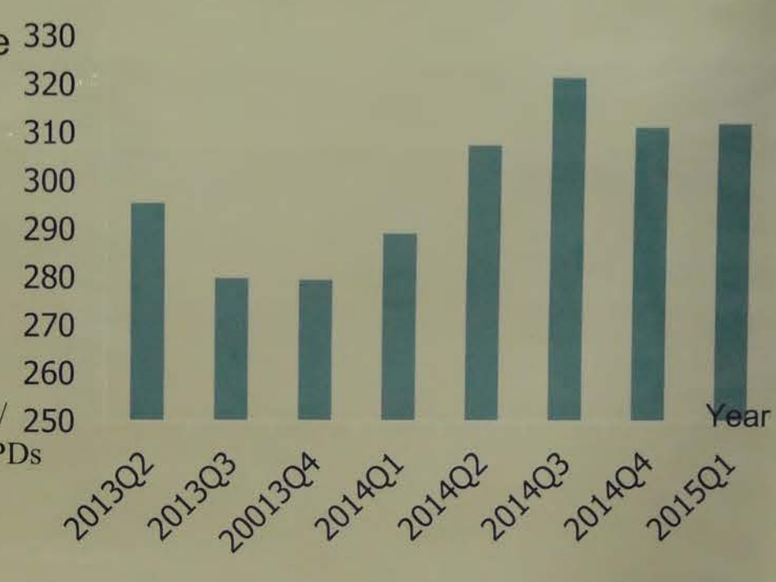
	Epithelial cell (LPF)	Neutrophils (LPF)
Group 1	>25	<10
Group 2	>25	10-25
Group 3	>25	>25
Group 4	10-25	>25
Group 5	<10	>25
Group 4 and 5 : accepted for cultures		

Results

In Stage I (Feb.2013-Feb.2014), the positive rate of sputum culture was 64.1% (2808/4381). In Stage II (Mar.2014-Mar.2015), the positive rate was 48.3% (2232/4620). It had excluded poor sputum specimen (16.9% , 780/4620).

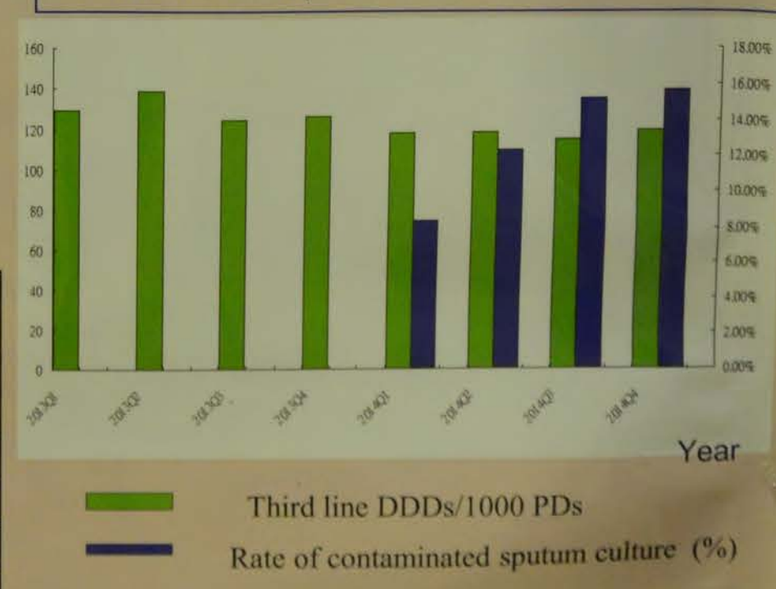
The difference of positive rates between these two stages was significant ($p<0.01$). The third generation antibiotics prescription amounts were 130 and 117.8 DDD/1000PD in Stage I and II, respectively. The third generation antibiotics usage in Stage I was significant higher than Stage II ($p=0.02$) (Table 2).. In the same time, the first generation antibiotics usage in Stage I was significant lower than Stage II ($p=0.01$) (Figure 1)

Figure 1. First line antibiotic prescription in 2013-2015



The rate of contaminated sputum specimen (>25 Epithelial cell/LPF) increased gradually after guideline initiated (March 2014), and the prescription amount of third line antibiotics gradually decreased (Figure 2). However, we found if we picked up the more contaminated sputum specimen, the inappropriate antibiotic usage was less.

Figure 1. Third line antibiotic prescription and rate of contaminated sputum culture



Conclusion

Evaluation of the quality of sputum specimen before culture performance is a good way to reduce inappropriate antibiotic prescription. It also reduce workload of microbiology laboratory staff.

Table 2. Amount of three lines of antibiotic prescription in 2013-2015

	2013Q2	2013Q3	20013Q4	2014Q1	2014Q2	2014Q3	2014Q4	2015Q1	p-value
First line DDDs/1000 PDs	295.508	279.965	279.609	289.303	307.652	321.766	311.533	312.441	**0.02**
Second line DDDs/1000 PDs	101.998	114.521	108.034	116.38	111.43	110.617	111.36	116.067	**0.44**
Third line DDDs/1000 PDs	129.197	139.373	124.946	126.469	117.87	119.088	115.032	119.181	**0.01**

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Microbiology

PA-47

Application of 16S rRNA metagenomics to investigate the bacterial community in northern Taiwan

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Introduction

Nowadays, transmission of healthcare-associated pathogens via surface contamination is now a critical issue in healthcare-associated infections. In this study, we applied a 16S ribosomal RNA (rRNA) metagenomics approach to investigate the bacterial community with two different cleaning methods at one medical intensive care unit (MICU) and one respiratory care center (RCC) at a hospital. MICU was performed terminal and daily cleaning, whereas RCC was performed terminal cleaning.

Material and Methods

A total of 72 samples, including 9 samples per sampling time, were analyzed. A high amount of microbial diversit was detected, with an average of 347 phylotypes per sample in MICU and an average of 328 phylotypes per sample in RCC.

Results

Human skin microbiota (HSM) was predominant in both wards compared to hospital environmental microbiota (HEM). In addition, the colonization rate of HSM in the MICU is higher than that in the RCC, especially Moraxellaceae. Net bacterial biomass was higher in non-cleaning areas compared to cleaning areas. In addition, the lack of daily cleaning in the RCC would contribute to the richness of bacterial communities.

Discussion

Comparing five different healthcare associated pathogens in the same ward, a significant higher abundance among Acinetobacter sp., Streptococcus sp., and Pseudomonas sp.. was shown in the RCC compared to the MICU by using paired-t test. This is the first in Taiwan to apply the 16S rRNA metagenomics to evaluate the effectiveness of cleaning approaches.

Figure 1、Compare sequencing method of Sanger and NGS



Figure 2、Reserved region sequences of 16S rRNA

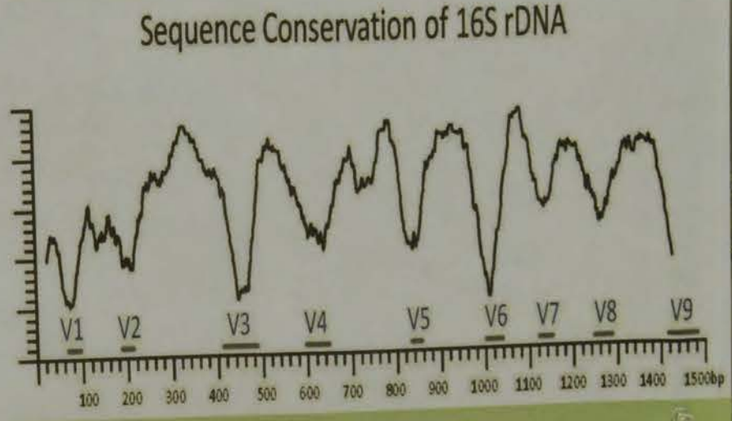


Figure 3、An area hospital ICU bacteria phase distribution

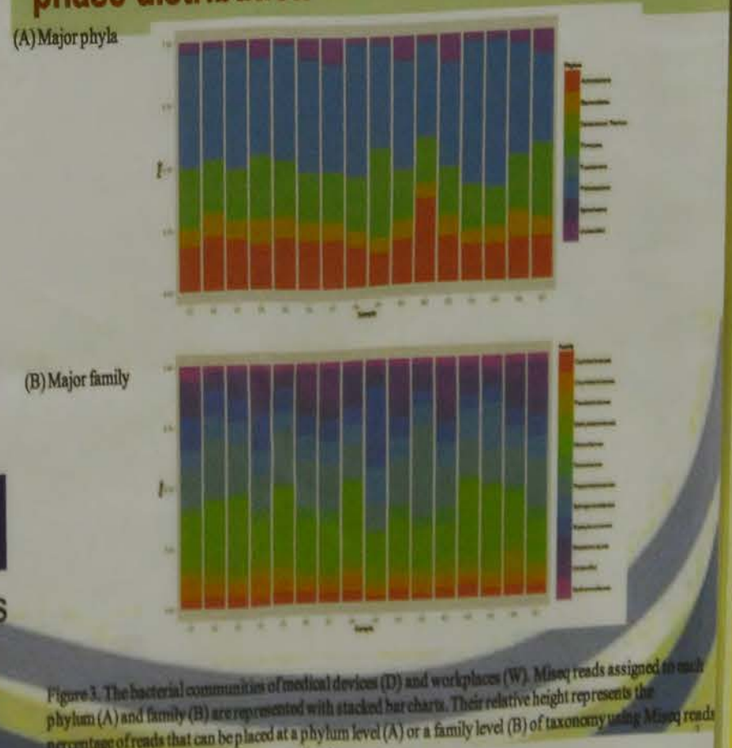
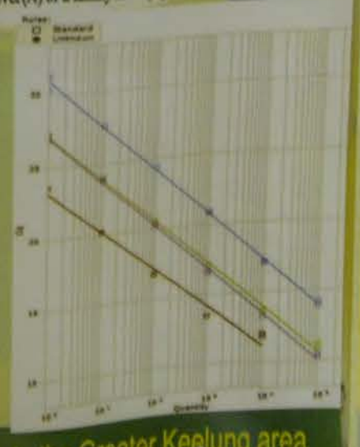


Figure 3. The bacterial communities of medical devices (D) and workplaces (W). Miseq reads assigned to each phylum (A) and family (B) are represented with stacked bar charts. Their relative height represents the percentage of reads that can be placed at a phylum level (A) or a family level (B) of taxonomy using Miseq reads with a BLASTX search of the Greengenes database.

Figure 4、A specific environmental standard curve of bacteria using Real-time PCR



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Microbiology

PA-48

PA-48

Consistency of Vitek-2, MALDI-TOF, and chromogenic agar for differentiation of vancomycin-resistant *Enterococcus faecium*

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Backgrounds

1. The chromogenic agar contains both the isolation and differentiation purposes.
2. The colony appearance on Chromagar VRE indicated that violet colonies are vancomycin-resistant *Enterococcus faecium* and green colonies are *E. faecalis.*
3. Neither *Enterococcus gallinarum* nor *E. casseliflavus* grow on Chromagar VRE.
4. Both Vitek-2 automatic identification system and MALDI-TOF MS system are widely applied for the identification of *Enterococcus* spp.



The green colonies of VRE on Chromoagar



The violet colonies of VRE on Chromoagar

Results

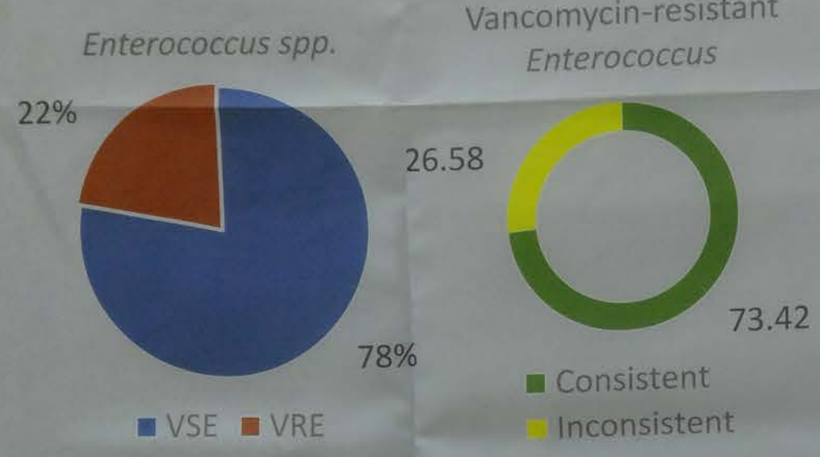


Figure 1: The percentages of VRE with inconsistent results between Vitek-2 automatic identification system and MALDI-TOF MS system.

Table 1 : The percentages of VRE in each cases of inconsistent results detected by Vitek-2 automatic identification system.

VITEK 2 Identification	Cases of VRE with inconsistent results			
	13	6	1	1
E. gallinarum	50%	34%	100%	0%
E. casseliflavus	50%	33%	0%	100%
E. faecium	0%	33%	0%	0%
Percent to total	100%	100%	100%	100%

Table 2 : The percentages of VRE in each cases of inconsistent results detected by MALDI-TOF MS system.

Identification MALDI-TOF MS	Cases of VRE with inconsistent results
	21
E. gallinarum	0%
E. casseliflavus	0%
E. faecium	100%
Percent to total	100%

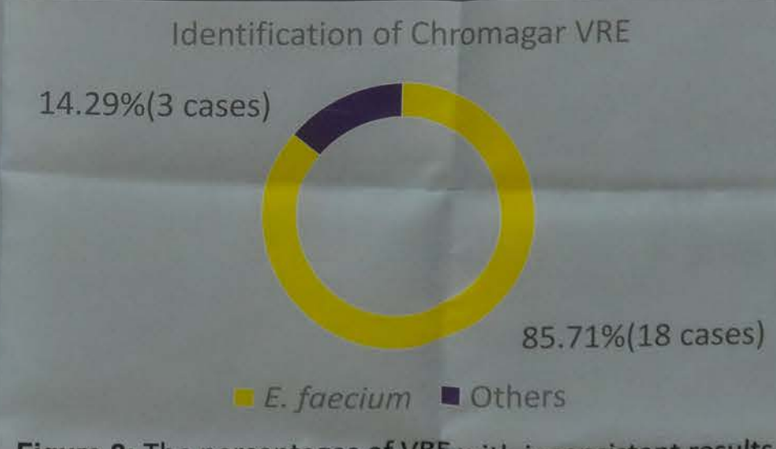


Figure 2: The percentages of VRE with inconsistent results detected by Chromogenic agar.

Conclusion

1. In conclusion, the results indicated that application of Chroagar VRE helps us to identify 86% (18/21) of inconsistent results of VRE.

Microbiology

PA-49

Comparison of *Pseudomonas aeruginosa* biofilm formation on soft contact lens and an effectiveness of contact lens care solutions

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The contact lens user has to be a significant risk factor for the development of microbial keratitis. The contact lens-related infections were led by the improper use of lenses and their cleaning process. The contact lens care solutions do not destroy all microbes, especially in the biofilm formation, even following instruction. The microbial communities in biofilm are surrounded with polymeric substances which increased resistance to antimicrobials and host immune responses. *Pseudomonas aeruginosa* is frequently encountered microbes that associated with contact lens-related infections. Such features make it resistant to some antiseptic including contact lens solution. Some studies reported that *P. aeruginosa* can cause keratitis in contact lens user due to the type of contact lens and poor quality of contact lens solution. This study compared the biofilm formation of *P. aeruginosa* on contact lens in each brand, and an effectiveness of contact lens solution on biofilm. The procedure started with culturing *P. aeruginosa* in Tryptic soy broth in microplate at 37°C for 24 hours and then tested with three brands of contact lens solution. This study also compared the reduction of the living-cells in biofilm after tested with contact lens care solution (CLCS) and control (PBS), in 2 to 3 log CFU reduction and 1 log CFU reduction, respectively. The only contact lens solution can damage biofilm structure but it could not against bacteria that living in biofilm structure. The results similarly showed the biofilm formation of *P. aeruginosa* on all tested contact lens. This study has demonstrated that CLCS was insufficient to prevent the contact lens-related infections on biofilm state.

INTRODUCTION

Contact lens is an invention for people who have visions problem such as nearsightedness, farsightedness, astigmatism or remedy of some corneal diseases. Moreover the contact lens is used for cosmetic reason. Fashion contact lens is easy to buy from general stores. The improper use of contact lens will be a cause of eye infection which makes ulceration on the cornea and can cause blindness. The infection causes by many factors such as users, solution of contact lens or a container of contact lens. Fungal , virus or bacteria could be infect to keratitis but the most common is from bacteria. This can be caused by Gram-positive and Gram-negative bacteria. *Pseudomonas aeruginosa* is a major causative of keratitis. This research studied an efficiency of contact lens solution to *P. aeruginosa* biofilm formation on contact lens.

METHODs and RESULTs

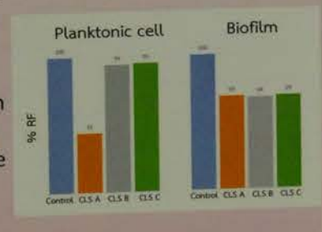
The effectiveness of CLCS to *P. aeruginosa*

Co-cultured 10^6 CFU/ml of *P. aeruginosa* with three different brands of tested CLCS → Incubated at 37°C for 6 hrs. → Planktonic state → Counted the living-cells

Cultured 10^6 CFU/ml of *P. aeruginosa* in TSB incubated 37°C overnight to form biofilm → Tested with three different brands of tested CLCS, Incubated at 37°C for 6 hrs. → Biofilm state → Counted the living-cells

A CLCS can damage biofilm structure but it could not against bacteria that living in biofilm structure.

The study of efficiency to planktonic stage shows that the same as CLCS which can damage biofilm is also against bacteria in planktonic stage while the others have the same results as above.



The living-cells of *P. aeruginosa* in biofilm on five tested contact lens are non significantly differences. We also compared the reduction of the living-cells in biofilm after tested with CLCS and control, in 2 to 3 log CFU reduction and 1 log CFU reduction, respectively.



The effectiveness of CLCS to *P. aeruginosa* biofilm on contact lens

The attachment of *P. aeruginosa* on five brands of contact lens
↓
Add 10^6 CFU/ml of *P. aerginosa* in 12-well plate with contact lens and incubate at 37°C overnight
↓
Count the living-cells in biofilm on contact lens by colony count

The effectiveness of CLCS to *P. aeruginosa* biofilm on contact lens
↓
After biofilm forms on contact lens, add in CLCS + 1% TSB, PBS + 1% TSB (control) and incubate at 37°C overnight
↓
Count the living-cells in biofilm on contact lens by colony count

CONCLUSIONs

There are non significantly differences in amount of lived cells in *P. aeruginosa* biofilm that attached on tested contact lens. This study shows that the living-cells in *P. aeruginosa* biofilm could not cleared by only using CLCS.

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ACKNOWLEDGEMENTs
This study was supported by a grant from Faculty of Allied Health Sciences, Chulalongkorn University.

PA-50

Effects of blood volume on blood culture results: a systematic review and meta-analysis

Chiao-Ni Wen[1,3], Hsiao-Chen Ning[1,3], Tsu-Lan Wu[2,3], Hsin-Yao Wang[1], Yueh-Lin Chung[1], Tsui-Ping Liu[1], Yu-Shan Wu[1,3], Jang-Jih Lu[1,3]

Department of Laboratory Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan City, Taiwan[1], Department of Medical Laboratories Administrative Center, Chang Gung Medical Foundation[2], Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan[3]

Abstract

Background

Blood cultures play a crucial role in the diagnosis of life-threatening bloodstream infection. Several studies have found that the adequate volume of blood drawn is the most important variable affecting positive rates of Blood cultures. Therefore, CLSI and many guidelines recommend an optimal volume of 20-30 mL for each set of blood bottles. However, many investigations based on cross-sectional studies just made before the existence of automated blood culture systems. There is the necessity to reassess the relationship of blood volumes with culture rates and other clinical variables after remarkable developments in blood culturing techniques in the past decade. Furthermore, in Taiwan, several hospitals could not meet criteria of the appropriate volume duo to some difficulties in sampling process. To deal with difficult situations, guidelines recommend at least 8 mL per blood culture bottle. In this way, we would like to evaluate the effectiveness of increasing the blood volume to improve the performance of blood culture.

Material and Methods

◆ Search strategy

✓Researchers: Chiao-Ni Wen and Yu-Shan Wu independently searched databases

✓Data source:
Primary database: Pubmed and EmBase in all languages published from Jan 1, 1950, to March 1, 2016
Secondary database: DynaMed and UpToDate

✓Keywords (MESH term) :

P	(	bloodstream	or	Bacteremia	Or	Sepsis / septic shock	) AND
I	(	blood culture	or	Volume	Or		) AND
C	(	blood culture	Or	Volume	Or		) AND
O	(	positive	Or	recovery rate	Or	positive rate	) AND

◆ Selection criteria

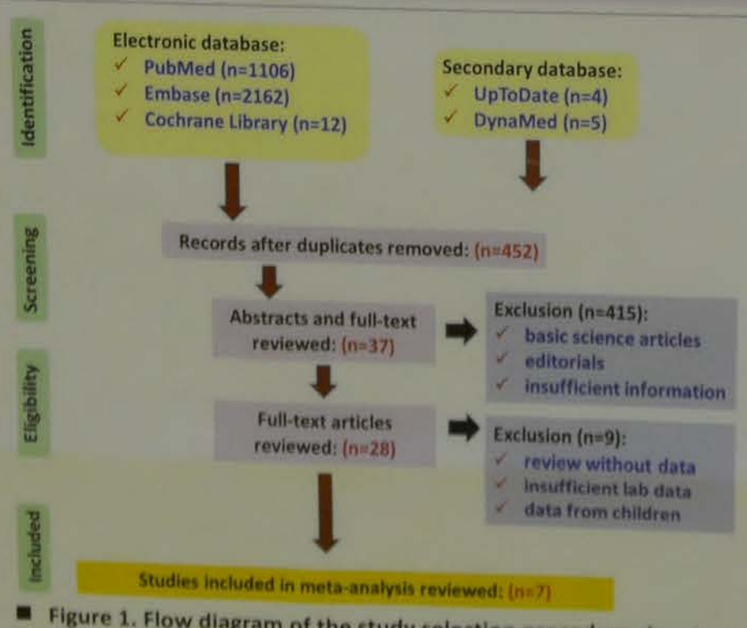
➢ Inclusion criteria:
- ✓ Study design: RCT, cohort, or cross-sectional study
- ✓ Comparison between different blood volumes and standard blood volumes were available
- ✓ Providing sufficient information on processing blood culture tests

➢ Exclusion criteria:
- ✓ Animal or experimental study
- ✓ Duplicate studies
- ✓ Lack of sufficient information on blood culture tests

◆ Quality Assessment
- ✓ Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2)

◆ Statistical analysis
- ✓ Comparisons between blood volume > 8 ml VS. < 8 ml : pooled OR by a random-effect model
- ✓ Heterogeneity test: forest plots, χ2 and I2 statistics
- ✓ Analysis: Medcalc Version 15.8 software

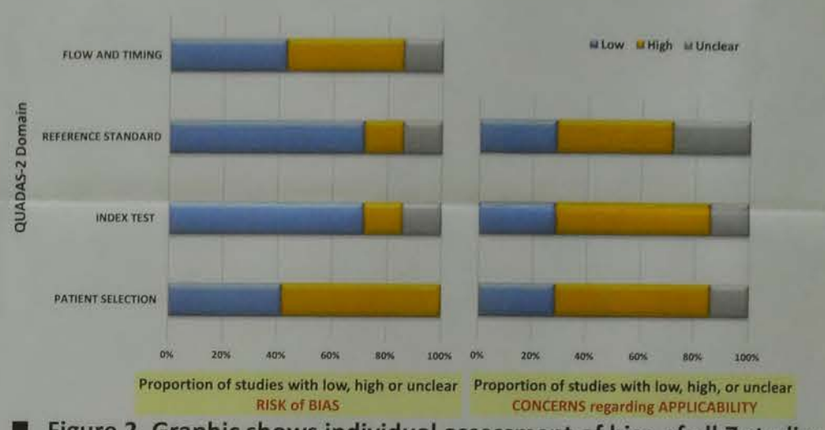
Results



■ Figure 1. Flow diagram of the study selection procedure showing the number of eligible studies for meta-analysis

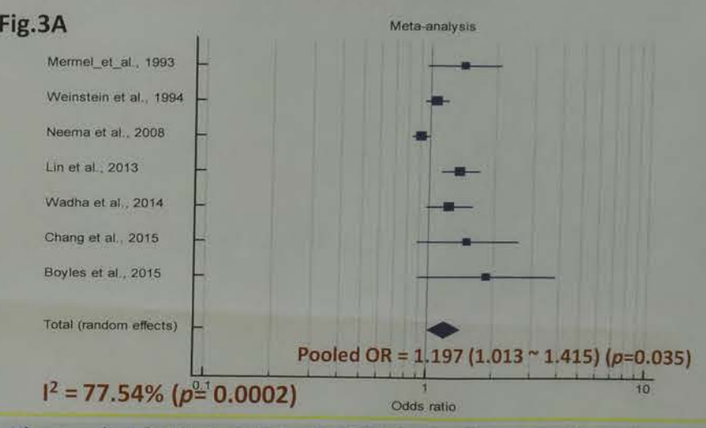
■ Table 1. Characteristics of studies included in systematic review and meta-analysis

Study	Publication year	Country	Study design	Study population	Sample size	Culture system
Mermel et al.	1993	USA	Cohort study	Adult patients	1658	NR-730 BACTEC
Weinstein et al.	1994	USA	Cohort study	Adult patients	13128	BacT/ Alert
Mtunthama et al.	2008	Malawi	Cohort study	Patients (undefined)	13370	BacT/ Alert
Lin et al.	2013	Taiwan	Cohort study	ER patients	4844	BACTEC 9240
Wadha et al.	2014	Kuwait	Cohort study	Patients (undefined)	5814	BACTEC
Chang et al.	2015	Korea	Cohort study	Adult patients	6048	BACTEC FX
Boyles et al.	2015	South Africa	Cohort study	ER patient	500	NA



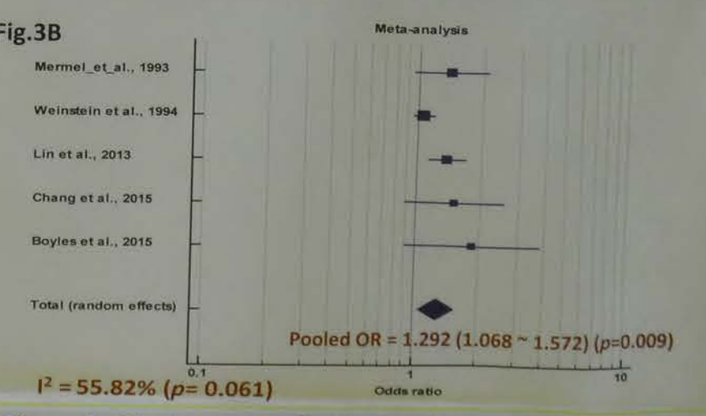
■ Figure 2. Graphic shows individual assessment of bias of all 7 studies using QUADAS-2 methodologic assessment tool

Fig.3A



◆ The pooled [OR]=1.197 (*p*=0.035). (< 8 ml VS. >8ml), and heterogeneity highly existed in the 7 studies (I^2=77.6% , *p*<0.001).

Fig.3B



◆ The evaluation heterogeneity improved tremendously after omitting 2 studies of "high risk of bias in patient selection" with undefined patient characteristics, and the pooled [OR]=1.292 (*p*=0.009).

■ **Figure 3. Forest plots of the pooled results for blood volume more than 8 ml VS. less than 8 ml.** 3a: Forrest plot to assess the pooled OR (reference: < 8 ml); 3b:Forrest plot to assess the pooled OR after omitting 2 studies of "high risk of bias" in patient selection.

Conclusion

- Reports concerning the importance of the volume of blood cultured with the new continuous-monitoring blood culture systems are scarce, and this is the first meta-analysis to evaluate the effectiveness of increasing the blood volume to improve the performance of blood culture between similar culture systems.
- The pooled estimates under the random-effects model suggested the greater the blood volume, the greater the culture rate in adults (> 8 ml blood volume VS.< 8 ml), pooled odds ratio [OR]=1.19, and there is true heterogeneity among these studies (I^2=77.6% , p<0.001).
- The inconsistency may arise through study populations with different underlying diseases.
- In conclusion, to backup the lab practice, further analysis will need to assess direct relationships between blood volumes and positive rates and time to positivity.

References

1. Mermel LA, Maki DG: Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. *Annals of internal medicine* 1993, 119:270-272.
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4. Lin HH, Liu YF, Tien N, Ho CM, Hsu LN, Lu JJ: Evaluation of the blood volume effect on the diagnosis of bacteremia in automated blood culture systems. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2013, 46:48-52.
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6. Chang J, Park JS, Park S, Choi B, Yoon NS, Sung H, Kim MN: Impact of monitoring blood volume in the BD BACTEC FX blood culture system: virtual volume versus actual volume. *Diagnostic microbiology and infectious disease* 2015, 81:89-93.
7. Boyles TH, Davis K, Crede T, Malan J, Mendelson M, Lesosky M: Blood cultures taken from patients attending emergency departments in South Africa are an important antibiotic stewardship tool, which directly influences patient management. *BMC infectious diseases* 2015, 15:410.

Microbiology

PA-51

Effects of Gram Staining Reports as soon as we get positive signal in Blood Culture

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INTRODUCTION

- In blood culturing, providing rapid isolation and identification of the causative organism, as well as providing prompt microbial sensitivity test results, is considered important for the best antimicrobial therapy.
- However, it can take about half a day to get those test results. It is because of this that we confirm the bacteria through Gram staining, and then use a 24-hour call system to inform the consulting physician as soon as possible once a positive blood culture is detected. This is because such immediate reporting of Gram stain results is thought to contribute to the evaluation of early antimicrobial therapy.

24-hour call system placed a burden

But in fact, 24-hour call system placed a burden on the clinical physicians receiving the calls.

Clinical physicians felt burdened by receiving calls after a patient's condition has stabilized or after the physician had gone home for the night.

Approach to reducing burden

We set up the **"<<Late Night>> call if positive"** item on the order system.

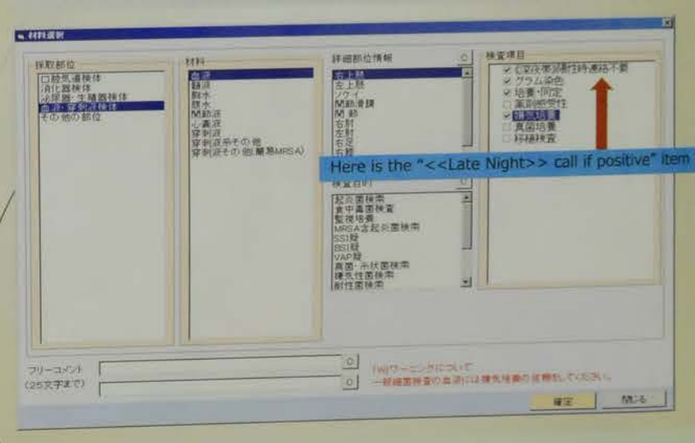
This method is in reference to physicians requesting the **"<<Late Night>> call if positive"** option and expressing the desire to receive Gram stain report's calls even in the late hours of the night.

AIM of This Study

1. In this study, we set up a **"<<Late Night>> call if positive"** item on the ordering system screen with the goal of reducing the burden. We also conducted a survey regarding the pros and cons of using the new item.
2. We investigated Gram staining result data recorded by clinical physicians on patient charts after the results were reported in order to evaluate the efficacy of immediate Gram stain reporting.

Display ordering system screen

This indicates the demand for the ability to request **"<<Late Night>> call if positive"** and receive immediate Gram stain reports regardless of time.



Survey Methods

Survey sheets were passed out to 161 regular physicians at this hospital's medical office, and then collected one week later. (Survey conducted in May of 2015)

Survey Request from the Diagnostic Department

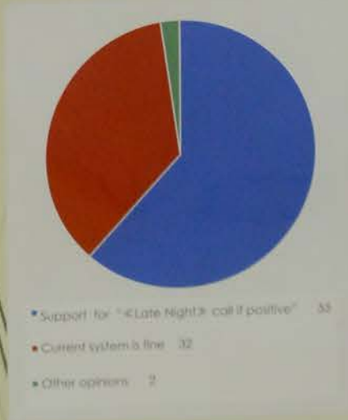
The Diagnostic Department informs physicians by telephone of Gram staining results when a positive blood culture is obtained. Excluding the Pediatric and Hematology Departments, which require 24-hour contact, we are examining the structure of late night (from around 10 PM to the following morning) telephone reporting. We have set up a "<<Late Night>> call if positive" item on the ordering system screen. Specifically, we are investigating the change to a reliable call method for physicians who request "<<Late Night>> call if positive" and wish to be contacted regardless of time. Please share your thoughts and requests with us.

(Please circle all that apply.)

( ) Only call me at night when I indicate "<<Late Night >> call if positive"
( ) The current system is fine.
( ) I'd like a second opinion if available.

Survey Results

Survey return rate was 55 % (89 / 161)



- 62 % (55 / 89) of physicians approved **"<<Late Night>> call if positive"**.
- 36 % (32 / 89) supported the current 24-hour immediate Gram stain reporting system.

Clinical Record Analysis Methods

- Among 2993 blood culture requests within the 6-month period from January to June 2016, 281 cases of immediate Gram stain reporting were considered.
- We calculated the proportion of cases that requested "<<Late Night>> call if positive".
- We examined the presence and details of any medical records related to the Gram stain results made within 12 hours of receiving the immediate Gram stain report call.

Clinical Record Results 1

34% (1022 / 2993) of the total blood culture orders requested **"<<Late Night>> call if positive."**

In the cases **"<<Late Night>> call if positive"** was requested,

- Patients were admitted through the emergency department.
- Patient's condition did not stabilize,… etc.

Clinical Record Results 2



- 63% (177 / 281) of cases that received immediate Gram stain reports mentioned the report in the medical records.
- Furthermore, 118 cases (42%) indicated an evaluation of antimicrobial drug being used.

Conclusive Words

- The surveys have shown that immediate Gram stain reporting was seen as an important diagnostic resource for early evaluation of antibiotic therapy. Because more than 40% cases received immediate Gram stain reports were confirmed to contain records related to evaluating current antibiotic therapy.
- We worked to set up the **"<<Late Night>> call if positive"** item on the ordering system, and then it had a effect to alleviate the burden on physicians who receive late night immediate Gram stain reports.

Microbiology

PA-52

The clinical impact of all-the-time-processing positive blood cultures

Junko Kawamura[1)], Mitsutaka Iguchi[2)], Tetsuya Yagi[2)],
Yukari Osada[1)], Hiroyuki Matsumoto[1)], Tadashi Matsushita[3)]

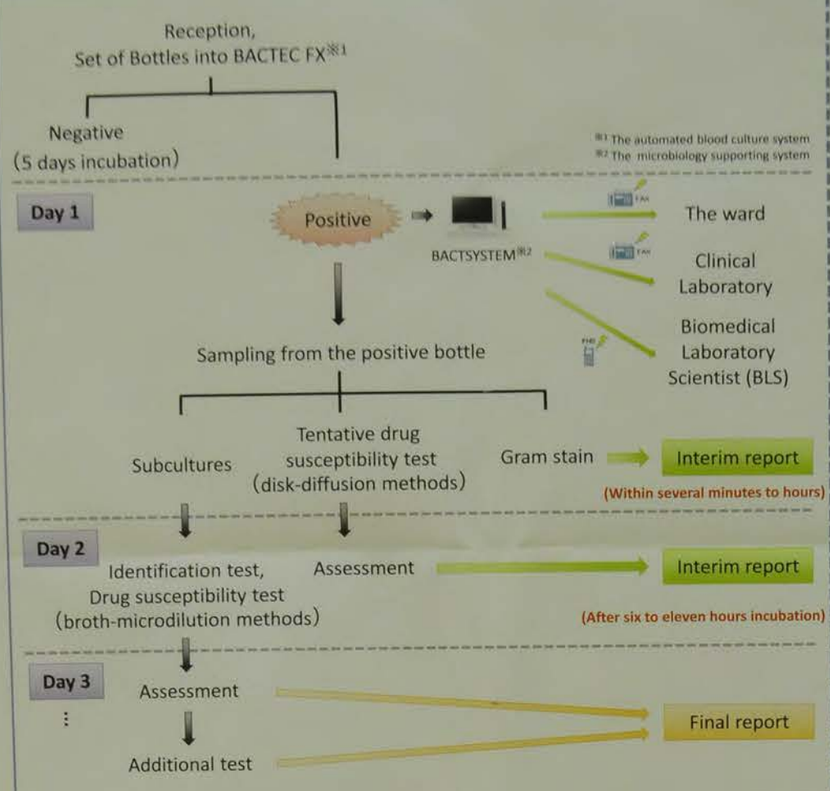
1) Department of Medical Technique 2) Department of Infectious Diseases
3) Department of Clinical Laboratory / Transfusion Medicine, Nagoya University Hospital, Japan

AIM To assess our activities toward all-the-time-processing positive blood cultures (PBCs).

BACKGROUND AND METHODS

All-the-time-processing PBCs are essential for providing early appropriate antimicrobial therapies, especially in sepsis or bacteremia. However, 24-hour-open clinical microbiology laboratory is very rare in Japan. In Nagoya University Hospital, although a lot of blood culture positive cases occurred on night shifts or holidays, the processing had been suspended until the next working day. We therefore had introduced all-the-time-processing (24-hour, 365-days) protocol in July 2006. In this study, we assess the clinical impact of this all-the-time-processing protocol by investigating the number of PBC events according to date or time, and the discordance between Gram stain (GS) findings and culture identification.

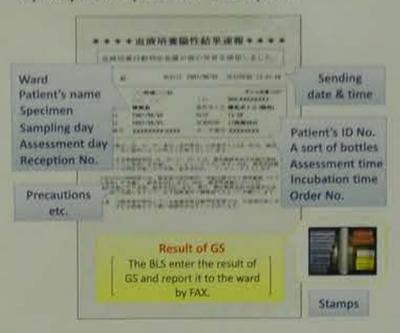
PROCESSING PROTOCOL



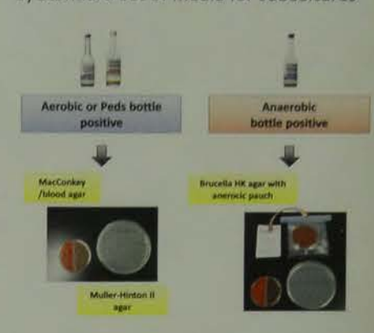
The tools of PBCs testing

We had prepared the manual containing the protocol.

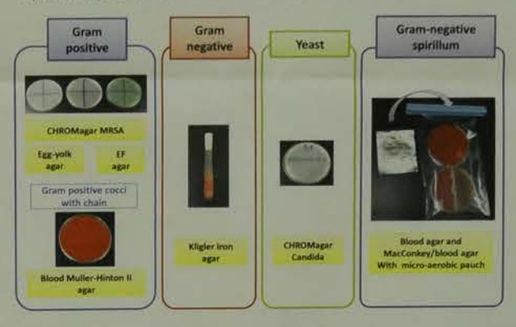
A) A quick report of PBCs by FAX



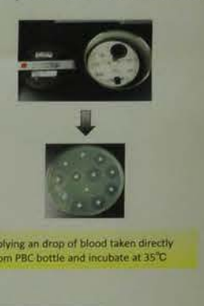
B) Standard set of media for subcultures



C) Additional set of media for subcultures



D) Tentative drug susceptibility test by disk-diffusion method



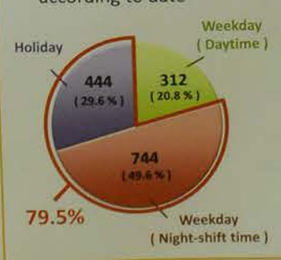
All non-microbiological and microbiological BLSs are involved in our all-the-time-processing PBCs after two half-day※3 training programs for GS, subcultures and tentative drug susceptibility test according to the manual.

(※3 They can experience the flow with about 10 cases.)

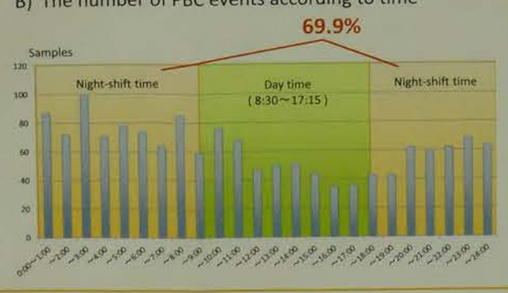
RESULTS 1: The number of PBC events

For the 12-month period, from April 2015 to March 2016, 1,500 (14.5%) of 10,338 sets became positive. Eleven (0.7%) of 1,500 sets were false-positive.

A) The number of PBC events according to date



B) The number of PBC events according to time



RESULT 2: Discordance between results of GS and culture identification

There were 26 (about 3.0%) cases of the discordant results between GS and culture identification. In 18 different error types, types 6, 7 and 11 occurred in each 2 cases, and type 15 in 6 cases.

	First reported result of GS	Revised result of GS	culture result	Cases
1	GPC (cluster)	GPC (chain)	*Enterococcus faecalis*	1
2	GPC (cluster)	GPC (chain)	*Aerococcus urinae*	1
3	GPC (twin)	GPC (chain)	*Streptococcus agalactiae*	1
4	GPC (twin)	GPC (chain)	*Enterococcus faecalis*	1
5	GPC	GPR	*Corynebacterium urealyticum*	1
6	GPR	GPC (chain)	*Streptococcus gallolyticus*	2
7	GPR	GNR	*Escherichia coli*	2
8	GPR	GNR	*Serottia marcescens*	1
9	GNR	GPR	GPR (suspected *Bacillus* sp.)	1

	First reported result of GS	Revised Result of GS	culture result	Cases
10	Yeast	GPC (cluster)	*Staphylococcus* sp. (suspected CNS)	1
11	undetermined	GPC (chain)	*Streptococcus mitis/oralis*	2
12	undetermined	GPC (chain)	*Streptococcus constellatus*	1
13	undetermined	GNC	*Moraxella* sp.	1
14	not detected	GPC (cluster)	*Staphylococcus aureus*	1
15	not detected	GNR (spirillum)	*Helicobacter cinaedi*	6
16	not detected	GNR (spirillum)	*Campylobacter jejuni*	1
17	not detected	Yeast	*Candida albicans*	1
18	not detected	Yeast	*Candida parapsilosis*	1

DISCUSSION

By reporting GS findings and processing subculture and tentative susceptibility test at night shifts, clinical microbiology lab could provide useful information to adjust initial antimicrobial therapy on the next morning of PBC events. This means that our all-the-time processing protocol enabled to shorten 24-48 hours for optimization of inappropriate therapy.

Although our system involved many BLSs without microbiological specialty, the interpretation of GS findings was considerably accurate owing to their prior trainings and the preparation of processing manuals. The most frequent errors were overlooking of spirillum because they were not familiar with this rare bacterium.

This collaboration of all member of BLSs brought the great contribution to the appropriate antimicrobial treatment for sepsis or bacteremia.

REFERENCES

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- Yoshinaga H *et al.*, *Jap Soci Clin Miclobiol*. 2010; 20(1): 20-27
- Asari S *et al.*, *Jap Soci Clin Miclobiol*. 2013; 23(Suppl. 1)

CONCLUSIONS

All-the-time-processing PBCs in collaboration of all BLSs in our hospital had favorable impacts to early appropriate treatment for sepsis or bacteremia.

Microbiology

PA-53

Antimicrobial susceptibility and *cfiA* carbamenemase gene positivity of *Bacteroides fragilis* group

Juntendo University Hospital, Department of Clinical Laboratory [1]
Juntendo University School of Medicine, Clinical Laboratory Medicine [2]

Manami Yamazaki[1], Ayako Nakamura[1], Masayoshi Chonan[1], Shigeki Misawa[1], Takashi Horii[1], Yoko Tabe[2], Akimichi Ohsaka[1]

Introduction

The *Bacteroides fragilis* group is responsible for the development of serious infections, such as intra-abdominal and bloodstream infections. A variety of drug-resistant strains that exhibit metallo-β-lactamase(MBL) production or metronidazole resistance have been reported, and the efficient monitoring of drug-resistance among this group, especially its resistance to carbapenems, is crucial for infection control in Japan.

1. Clarify the state of resistance detection in the *B. fragilis* group
2. Reveal a valid method for detecting carbapenem-resistant strains

Materials & methods

【Review period】 Strains isolated from clinical materials (except stools) at Juntendo University Hospital from January 2013 to October 2014

【Isolates】 Detected using Anaero Columbia rabbit blood agar (BD)
Bacteroides fragilis (83 strains) *Bacteroides thetaiotaomicron* (28 strains)
Bacteroides vulgatus (9 strains) *Parabacteroides distasonis* (9 strains)
Bacteroides ovatus (8 strains) Six other species (13 strains)

【Identification】 Identification was performed using the MALDI Biotyper (Bruker Co. ltd.)

【Susceptibility testing】 Kyokuto MIC plates (Kyokuto co. ltd.) according to the Clinical and Laboratory Standards Institute (CLSI) performance standards (M100-S24)

Examined antimicrobial agents : piperacillin(PIPC), PIPC/tazobactam(TAZ), ampicillin/sulbactam(ABPC/SBT), cefmetazole(CMZ), imipenem(IPM), meropenem(MEPM), clindamycin(CLDM), minocycline(MINO), moxifloxacin(MFLX) and metronidazole(MNZ)

【Detection of the carbapenemase gene】 The *cfiA* gene, which encodes carbapenemase, was detected using the polymerase chain reaction(Soki's method)
Primer Forward: 5'- TCC ATG CTT TTC CCT TGC GCA GTT AT -3'
Reverse: 5'- GGG CTA TGG CTT TGA AGT GC -3'

【Carbapenemases confirmation tests】

1) Enzyme inhibition test using sodium mercaptoacetate (SMA)
Anaero Columbia agar was inoculated with the test strain. Then, imipenem and meropenem disks were placed 3 cm apart from each other on the agar. Filter paper soaked with SMA was placed between the disks, and the bacteria were cultured for 24 hours at 35℃. The target strain was considered to be MBL-positive if a growth inhibition zone was observed around the imipenem or meropenem disk.

2) Carbapenemase inactivation method (CIM)
The test strain was inoculated into 400 μL sterile water, incubated for 2 hours in the presence of a meropenem disk. *E. coli* ATCC25922 were added to the Anaero Columbia agar, the meropenem disk was removed, and the plate was incubated for 8 to 12 hours. If no growth inhibition zone was seen, the test strain was considered to be carbapenemase-positive.

Results

1. The *B. fragilis* group, exhibited PIPC/TAZ, ABPC/SBT, IPM, MEPM, and MINO resistance rates of <10%. All strains were suscepyible to MNZ.
2. The effects of PIPC and CMZ differed among the bacterial species, e.g., *B. fragilis* was susceptible to these agents, whereas *B. thetaiotaomicron* was resistant to them.
3. The *cfiA* gene, which encodes carbapenemases, was detected in *B. fragilis*, which was MBL-positive (the SMA enzyme inhibition test produced a positive result). In *B. fragilis*, the MIC of MEPM was ≧4 μg/mL.
4. The carbapenemase-producing strain exhibited higher MIC values for MEPM than for IPM. The MBL test was easy to perform with MEPM.
5. While some strains other than *B. fragilis* demonstrated carbapenem resistance, *cfiA* was not detected in these strains, and they were MBL-negative.

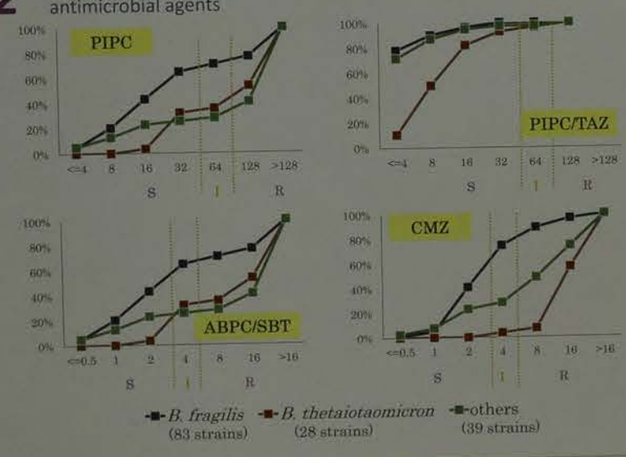
result **1** Susceptibility of the *Bacteroides fragilis* group to antimicrobial agents

N = 150

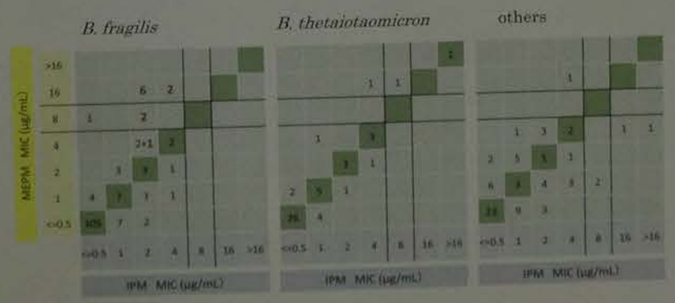
antibiotics	MIC (μg/mL) ≦0.5	1	2	4	8	16	32	64	128	>128	% of resistant	MIC_{80}
Piperacillin				2	5	36	31	21	30	25	36.7%	128
Piperacillin/tazobactum				96	27	17	7	1	2		1.3%	8
Ampicilline/sulbactum			62	21	27	31	7	2			6.7%	16
Cefmetazole			2	5	36	31	21	30	25		36.7%	64
Imipenem	83	30	23	10	3		1				0.7%	2
Meropemem	92	23	15	7	3	9	1				6.7%	2
Clindamycin	28	23	12	14	1		72				48.7	>32
Minocycline	30	8	55	46	7	4					2.7%	4
Moxifloxacin	33	32	17	11	26						24.6%	8
Metronidazole		49	71	30							0.0%	2

intermediate

result **2** Comparison of the susceptibility of *Bacteroides* species to various antimicrobial agents

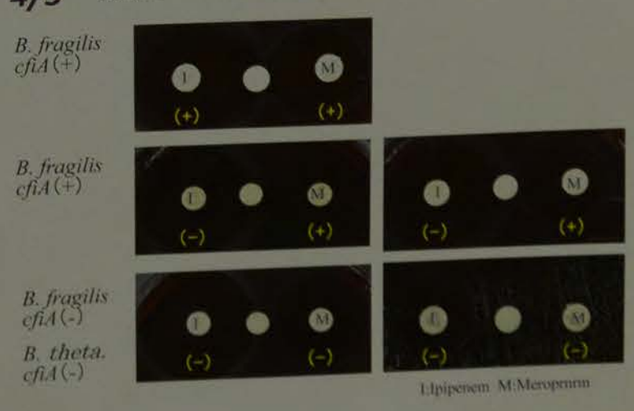


result **3** Correlation between the MIC distributions of IPM and MEPM



The numerical values shown in red is the *cfiA* gene-positive strains

result **4/5** Enzyme inhibition test using SMA



result **4** Characterization of the IPM/MEPM resistance of *B. fragilis* (MIC≧4μg/mL)

Species	ABPC/SBT	PIPC	PIPC/TAZ	CMZ	IPM	MEPM	*cfiA*	CIM	MBL MEPM	MBL IPM
B. fragilis	16/8	32	16/4	64	4	16	+	–	+	+
B. fragilis	16/8	32	8/4	32	2	16	+	–	+	–
B. fragilis	16/8	32	8/4	32	2	16	+	–	+	–
B. fragilis	16/8	32	≦4/4	32	2	16	+	–	+	–
B. fragilis	8/4	>128	≦4/4	32	2	16	+	–	+	–
B. fragilis	16/8	32	16/4	32	2	16	+	–	+	–
B. fragilis	16/8	32	16/4	64	2	16	+	–	+	–
B. fragilis	16/8	32	16/4	64	4	16	+	–	+	+
B. fragilis	32/16	32	16/4	64	4	16	+	–	+	–
B. fragilis	16/8	>128	≦4/4	32	2	8	+	–	+	–
B. fragilis	16/8	32	32/4	32	2	8	+	–	+	–
B. fragilis	8/4	8	≦4/4	4	≦0.5	8	+	–	+	–
B. fragilis	8/4	8	≦4/4	16	2	4	+	–	+	–
B. fragilis	16/8	>128	8/4	16	4	4	–	–	–	–
B. fragilis	8/4	>128	≦4/4	>64	2	4	–	–	–	–
B. fragilis	16/8	>128	8/4	>64	4	8	–	–	–	–
B. fragilis	8/4	>128	≦4/4	>64	4	8	–	–	–	–
B. fragilis	16/8	>128	≦4/4	8	4	4	–	–	–	–

result **5** Characterization of the IPM/MEPM resistance of strains other than *B.fragilis* (MIC≧4μg/mL)

Species	ABPC/SBT	PIPC	PIPC/TAZ	CMZ	IPM	MEPM	*cfiA*	CIM	MBL MEPM	MBL IPM
B. thetaiotaomicron	64/32	>128	>128/4	>64	>16	>16	–	–	–	–
B. thetaiotaomicron	32/16	>128	128/4	>64	8	16	–	–	–	–
B. thetaiotaomicron	8/4	>128	8/4	64	4	8	–	–	–	–
B. thetaiotaomicron	16/8	>128	32/4	64	4	4	–	–	–	–
B. vulgatus	32/16	>128	≦4/4	4	8	1	–	–	–	–
B. vulgatus	16/8	>128	≦4/4	≦2	8	1	–	–	–	–
B. ovatus	16/8	>128	≦4/4	64	4	4	–	–	–	–
P. distasonis	32/16	>128	≦4/4	32	4	1	–	–	–	–
P. distasonis	16/8	>128	16/4	>64	2	4	–	–	–	–

Conclusion

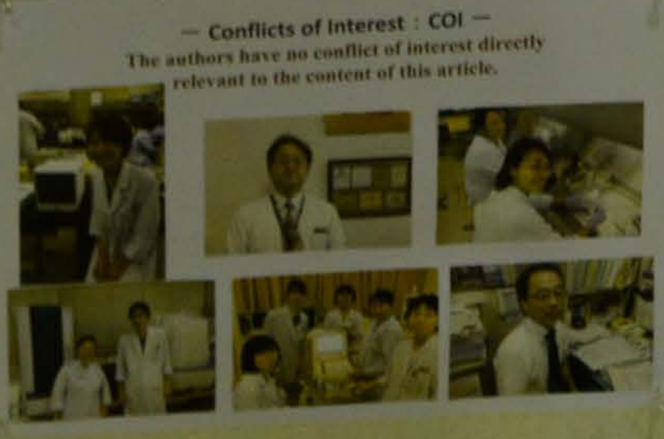
Carbapenemase-producing *B. fragilis* can be screened for based on the MIC of MEPM (≧4 μg/mL), by detecting the *cfiA* gene, or by performing the SMA test using MEPM. Carbapenem resistance in other *Bacteroides* species is considered to be due to factors other than carbapenemases.

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— Conflicts of Interest : COI —

The authors have no conflict of interest directly relevant to the content of this article.



Microbiology

PA-54

Endoscopy-related infection control using the microbiological monitoring

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[2]Osaka University Graduate School of Medicine

Background: Diagnostic procedures using endoscopy have markedly progressed, enabling precise diagnoses and minimally invasive treatments. The number of endoscopic examinations and treatments consequently increases every year. However, there are reports of infection via the endoscope; therefore, a system of safe and advanced endoscopic procedures is required. At our laboratory, we have been regularly monitoring the cleanliness of endoscopes managed by the endoscopy center since 2009 using microbial stain and culture methods to control endoscopy-related infections.

Methods: Checked samples were frequently used in routine tests.

① the tip of an endoscope immersed in sterile saline (15~20mL)
② draw sterile saline through the biopsy channel
③ flush into the biopsy channel
④ repeat ①~③15 times
⑤ the saline was collected in sterile containers
⑥ sample was centrifuged and the pellet was stained and cultured
- Gram stain, Ziehl-Neelsen stain
- Cultivation for Bacteria and Mycobacteria





Fig. 1 Methods of endoscope microbiological monitoring

Table 1 Criteria of endoscope microbiological monitoring

Level	Stain	Culture	Result	Comments
1	Negative	Negative	Clean	–
2	Positive	Negative	Semi clean	–
3	Negative	Positive	Unclean	Can not use / Re-test / Need of maintenance
4	Positive	Positive	Dirty	

Endoscopes that were determined to be contaminated by the endoscope cleanliness monitoring were retested after cleaning and disinfection.

second test were requested maintenance from the manufacturer.

Table 5 The main bacteria detected from endoscope

sample	Organism
gastroscope	*Pseudomonas aeruginosa, Klebsiella pneumoniae Candida* spp., Nonfermentive Gram-Negative Bacilli
colonoscope	*Enterococcus* spp., *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*

- environment of the endoscope cleaning room — no problem
- environment of the endoscopy center — no problem
- automatic endoscope reprocessor — no problem
- cleaning and disinfection process for endoscopes — improve
- questionnaire to the endoscope cleaning staff — improve process & setting of the manual
- inside of endoscope channel — request maintenance

The probability that bacterial contamination remains after cleaning and disinfection is high.



Fig. 3 Surveillance for the cause of contamination

Results: From 2009 to 2015, we examined 180 gastroscopes,199 colonoscopes, and 253 bronchoscopes. Forty-two gastroscopes and 29 colonoscopes tested positive.



Fig. 2 Positive plate

Table 2 Culture results by gastroscope

	2009	2010	2011	2012	2013	2014	2015	Total
Number of tests	40	23	24	22	25	22	24	180
Number of positive	0	4	8	8	11	7	4	42
Positive rate(%)	0	17.4	33.3	36.4	44.0	31.8	16.7	23.3

Table 3 Culture results by colonoscope

	2009	2010	2011	2012	2013	2014	2015	Total
Number of tests	47	36	24	22	24	22	24	199
Number of positive	6	9	2	4	7	1	0	29
Positive rate(%)	12.7	25.0	8.3	18.2	29.2	4.5	0	14.6

Table 4 Culture results by bronchoscope

	2009	2010	2011	2012	2013	2014	2015	Total
Number of tests	40	30	40	36	36	34	37	253
Number of positive	0	0	0	0	0	0	0	0
Positive rate(%)	0	0	0	0	0	0	0	0

Ten gastroscopes and 3 colonoscopes that were determined to be contaminated by the

Discussionon: Many organizations recommend microbiological surveillance of endoscopes, but some do not. Because endoscope contamination was confirmed, despite using an automatic endoscope reprocessor (AER), it is important not to overestimate the cleaning and disinfection effectiveness of the AER.

Table 6 Microbiological surveillance by different guidelines

Guidelines (year)	Sampling method	Sampling site	Surveillance frequency
APIC (2000)	Flushing, brushing	Suction/biopsy, air/water, elevator and carbon dioxide channel	Routine test not recommended
ESGE-ESGENA (2008)	Anterograde flush: channels Swabs: outer surfaces Liquid samples: water bottles	All channels Outer surfaces Connected water bottle	No longer than 3 months
BSG (2008)	For atypical mycobacteria Rinse water: AER	AER	Annual
GESA-GENCA (2010)	Flush-brush-flush: channels Anterograde sampling supported by retrograde sampling Flushing with filter: AER Liquid samples: water bottles	All channels AER Water for manual rinsing or water supply to AER	AER, duodenoscopes: every 4 weeks Other GI endoscopes: every 3 months
CDC (2015)	Brush: distal end of the duodenoscope Anterograde flush: channels	Instrument channels and distal end of the duodenoscope	Routine test not recommended
JSIPC(2010)			Routine test not recommended

APIC:Association for Professionals in Infection Control and Epidemiology, ESGE-ESGENA:European Society of Gastrointestinal Endoscopy and European Society of Gastroenterology and Endoscopy Nurses and Associates, BSG:British Society of Gastroenterology, GESA-GENCA: Gastroenterological Society of Australia and Gastroenterological Nurses College of Australia, CDC:Centers for Disease Control and Prevention, JSIPC:Japanese Society for Infection Prevention and Control, AER:automatic endoscope reprocessor

Conclusion: Monitoring of endoscope cleanliness performed by the microbiology laboratory can prevent nosocomial endoscopy-related infections and be useful in determining the timing of endoscope maintenance.

Microbiology

PA-55

Epidemiological chase of MRSA using POT method in NICU and GCU for 20 months

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Background

Newly isolated MRSA strains in NICU (Neonatal Intensive Care Unit) and GCU (Growing Care Unit) were analysed by using the PCR based open reading frame typing (POT) method. This method is one of the molecular epidemiological methods. The procedure of POT is quite easier than PFGE, whereas its resolution power is similar to that of PFGE. Here we report the POT analysis data of MRSA in a long period of 20 months.

Outline of NICU and GCU in our hospital

number of beds : 31 (NICU:18, GCU:13)
- Pediatrics : 25
- Pediatric surgery : 6

number of pediatricians : 7
Number of patients per pediatrician : 6-9
number of nurses : 56 (NICU:34, GCU:22) , 2-shift system



Methods

- **Isolates**

  A total of 82 MRSA strains (NICU:67,GCU:15) were collected from May 2014 to December 2015.

- **Genetic analysis for epidemiology**

  A Cica Geneus Staph POT KIT (Kanto Chemical) was used for this analysis.

1. Culture strains at 37℃ overnight on Mueller Hinton Agar plates.
2. DNA Extraction (15min)
3. Multiplex PCR (2h30min)
4. Agarose gel electrophoresis (60min)
5. Read electrophoretic banding patterns.
6. Using a result of having this electrophoretic band or not, and input it into a calculation sheet for POT type analysis by the binary scale and calculate a POT type of three categories.
7. Compare the homogeny

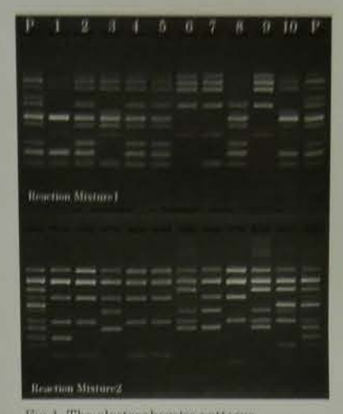


Fig.1. The electrophoretic patterns

P : Positive control 2,4,5 : Same POT type (160-183-34)
1-10 : Clinical isolates 8,9 : Same POT type (108-136-112)

References : Manual of CicaGeneus™ Staph POT KIT, Kanto Chemical

Results

Table 1. POT type and number of isolates

POT type	Total	NICU	GCU
106-183-34	29	23	6
106-9-80	14	13	1
106-247-33	6	3	3
106-9-2	6	5	1
69-221-111	4	4	0
106-137-80	4	2	2
108-136-112	3	3	0
98-137-15	2	2	0
106-183-37	2	2	0
98-147-98	2	2	0
106-27-104	2	2	0
Others	8	6	2

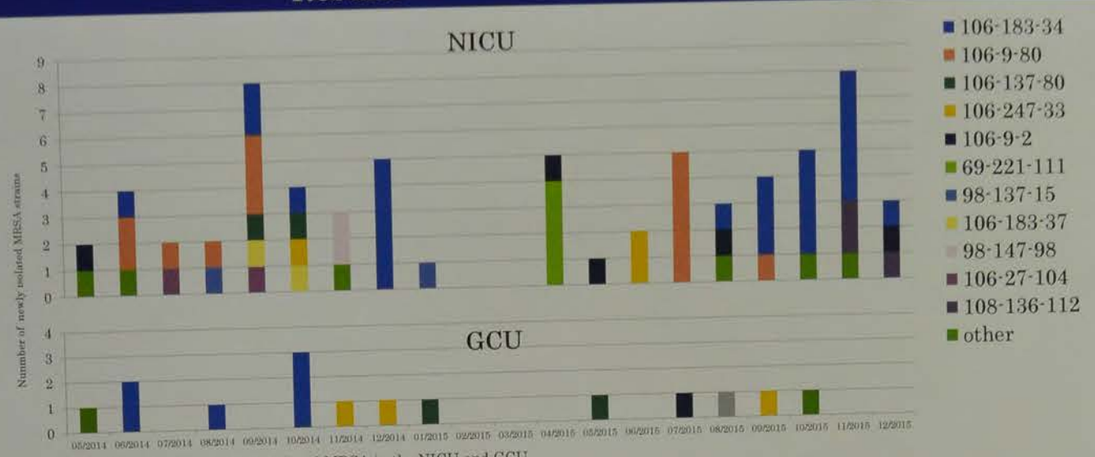


Fig.2. The trend of the newly isolated MRSA in the NICU and GCU.

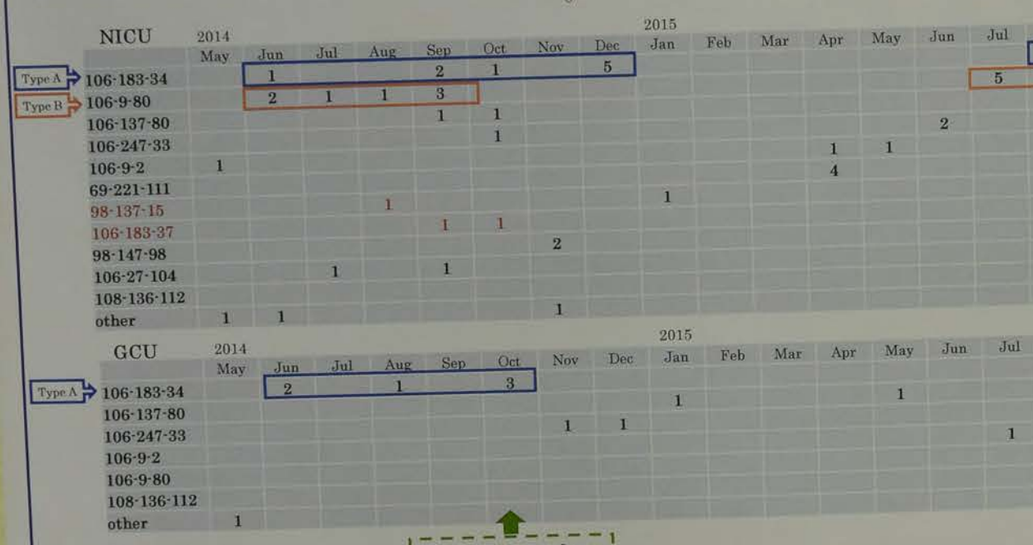


Fig.3. Number of newly isolated MRSA strains in the NICU and GCU.

2 types of strains (106-183-37, 98-137-15 : red text in Fig.3.) with the same POT types were isolated from stethoscope earpieces of the hospital staffs. From these results, we started to disinfect the stethoscope earpieces everyday and strengthened the education of hand hygiene.



Disinfection of stethoscope earpieces

- ✓ Wipe the stethoscope with 70% ethanol before and after use.
- ✓ Wipe the tube of the stethoscope in one direction and also wipe the earpieces.
- ✓ The stethoscope is immersed in the sodium hypochlorite solution for one hour after use.

The same POT type strain has not been detected afterward.

Results and Discussion

- One POT type consists of a combination of three POT numbers, i.e. POT1-POT2-POT3.
- All isolates were classified into 19 POT types.
- There were 2 major strains, type A (POT number 106-183-34, 29 strains, 35%) and type B (POT number 106-9-80, 14 strains, 17%).
- Type A strains were detected from multiple patients in NICU and GCU at July 2014, and continued to be detected sporadically over following 6 months in NICU and 4 months in GCU. The strains have not been detected for 7 months after January 2015. It was detected again in August 2015 and continued to be detected during another 5 months in NICU. That is, type A strains continued to be detected sporadically during the 18 months.
- Type B strains were detected in succession during four months in NICU. The type B strains disappeared once, but were detected again 10 months later.
- This method was useful to clarify that the isolation of both strains intermittently lasted for long periods.
- Sixty eight strains (83%) had the same POT1 number 106. Most strains of POT1 number 106 are supposed to have SCC*mec* IV gene, which is popular among community-acquired type of MRSA strains. Contrary to these characteristics, most patients in our hospital were hospital-acquired. That means even community-acquired type of MRSA may become prevalent in hospital environment.

Conclusion

This 20-month-study revealed that two strains were predominantly detected for the long term from NICU in our hospital. The PCR based results are transformed into a three-part of POT numbers. So it is quite feasible to compare clinical strains isolated over time and also compare clinical and environmental strains. The POT method is a useful epidemiological method for estimating the transmission route and eventually for infection prevention.

Microbiology

PA-56

Moleculare epidemiological analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) by PCR-based ORF Typing (POT) method

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Background

Multilocus sequence typing (MLST) and Pulsed-field gel etectrophoresis (PFGE) have been widly used in epidemiological analysis of MRSA. Howere, it is very difficult to perform them in the community hospital. Recently, a new epidemiological analysis of MRSA, polymerase chain reaction (PCR)-based ORF typing (POT) method, was developed[1].

In this study, we performed a molecular epidemiological analysis of MRSA isolated from the skin and soft-tissue using the POT method. And, we were also investigated possession of Panton-Valentine leukocidin (*lukS/F*-PV) gene.

Material and Methods

We used the MRSA 90 strains, which were isolated from skin and soft-tissue at National Hospital Organization Nagasaki Kawatana Medical Center between 2005 and 2011. We analyzed the strains by Cica Geneus Staph POT-kit (Kanto Chemical co., Tokyo, Japan). The POT method could classify the MRSA strains by analyzing the pattern of detected amplified bands, and based on the POT1-value, clonal complex (CC) type and SCC *mec* type could be estimated. And The operating time is approximately 4 hours. Additionally, we detected a Panton-Valentine leukocidin (*lukS/F*-PV) gene by real-time PCR as by previously reported[2].

Results

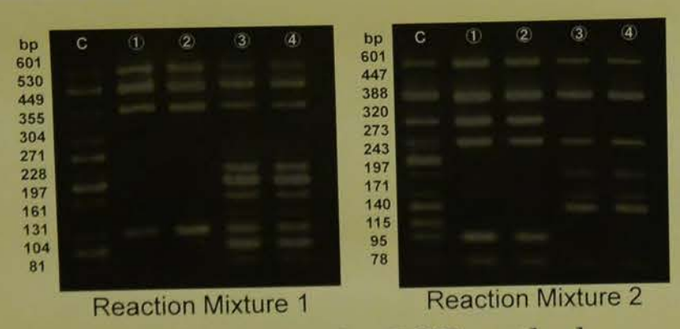


Figure1. Genotyping by POT method

POT method detected the 22 amplification products using the PCR, and these products were classified into POT1, POT2 and POT3. Sample 1 and 2 were identified as POT 110-4-37 (POT1 : 110, POT 2 : 4, POT 3 : 37), and they were considered as the same strain. Sample 3 and 4 were identified as POT 116-127-113, and they were considered as the same strain.

Results

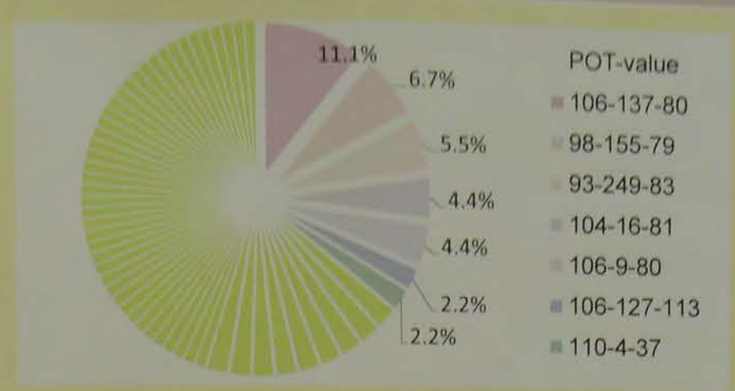


Figure 2. The POT-value of MRSA 90 strains

We calculated the POT-value according to the manufacturer's instructions. The POT-value of MRSA 90 strains were classified into 59 patterns. The most common POT-value (106-137-80) was detected in 10 strains (11.1%), and the second most common POT-value (98-155-79) was detected 6 strains (6.7%). The *lukS/F*-PV was detected in 4 strains, and their POT-value were 106-127-113 (2 strains) and 110-4-37 (2 strains). Based on the POT-value, they were differ from USA-300 strain.

Table 1. The clonal complex type (CC) and SCC *mec* type estimated by POT1-value

POT1-value	Number of strain	CC	SCC *mec*
106	39	1 or 8	Ⅳ (most USA300 strains were; 106-77-113)
93	22	5	Ⅱa (NY/ Japan)
98	10	8	Ⅰ
104	6	12 or 56 or 59 or 72	Ⅳ
64	1	59 or 89	Ⅴ
other	10	-	-

Based on the POT 1-value, the most common strains were estimated as CC1 or 8 and SCC *mec* type IV, and the second common strains were CC5 and SCC *mec* type IIa.

Conclusion

The POT method could classify the MRSA strains and estimate the MRSA clone. Since the operating time is short (approximately 4 hours), the POT method can contribute to the quick determination of MRSA outbreak.

Reference

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athogenic bacteria in feces
ippine and Nepal

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atory, Mie University Hospital
Science and Technology & Nat' Inst of Tropical Medicine and
niversity of the Philippines Los Banos
Health University

Purpose

has inferior public health environment. In this environme
people are infected with diarrhea disease, especially enter
dren. The aim of this study to investigate pathogenic bacte
epal.

Methods

years school children were collected in these countries and
nilippine school children and 150 samples of Nepal school
his study was *Vibrio cholera*, *Vibrio parahaemolyticus*, *Sal*
us aureus and diarrheagenic *Escherichia coli*. In addition, fe
ned for drug-resistant (KPC, MRSA and ESBL).

Fecal samples

BGLB
TCBS
SS
DHL
Detection of *Vibrio* spp.
Detection of *Salmonella* spp.
ification of *E.coli*
or *Klebsiella* spp.
diffusion susceptibility testing

Results

nic *E.coli* by PCR

Table 2: Detected as

Detection numbers (n=70)	No. of samples	*mec* A gene
0	12	+
0	17	+
0	35	+
0	40	+
5	63	−
0	108	+
0		
3		
62		

of *E.coli* with drug
ity testing

Table 4: Detection of ESBL of *Klebsiella*
diffusion susceptibility testing (K

CTX	CTX/CVA	CAZ	CAZ/CVA
34	33	22	38
38	35	25	36
30	30	24	32
32	30	15	30
25	30	22	30
28	30	20	28

Discussion

e easily get some antibiotic without medical prescription. Theref
acteria increasing than Japan whose system require medical pres
ve demonstrated that a carrier rate of ESBL with drug-resistant a
ool children and Japanese. Additionally, KPC- producing bacteri
dy is contributed to be improvement of public health because th
detail and for international requirement among Japan, Philippine

Microbiology

PA-57

Development of a novel matrix-assisted laser desorption/ionization time-of-flight mass spectrum (MALDI-TOF-MS)-based typing method to identify meticillin-resistant *Staphylococcus aureus* clones

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Background

Matrix-assisted laser desorption/ionization time-of-flight mass-spectrum (MALDI-TOF MS)-based strain typing for microorganisms is a fast and inexpensive technology for identification of bacteria. Mass spectrum analysis enables species- and subspecies-level identification, and can be used as an epidemiological tool in outbreak management. However, its reliability at clonal level identification has not been well established yet. We have already reported that MALDI-TOF MS has almost equivalent performance for the clonal identification as pulsed-field gel electrophoresis (PFGE) and phage open-reading frame typing (POT). We report further investigation result for the capability of MALDI-TOF MS for the clonal identification of the methicillin-resistant *Staphylococcus aureus* (MRSA) using repetitive element palindromic polymerase chain reaction (rep-PCR) analysis.

Samples and methods

The standard *S. aureus* strain (ATCC 29213) and 57 clinical isolates of MRSA, which were randomly selected from 231 MRSA clinical isolates at Saga University Hospital in 2013, were used for MALDI-TOF MS analysis in this study. Then, POT and rep-PCR measurements were performed. The results of MALDI-TOF MS, POT and rep-PCR were compared with other 24 MRSA clinical isolates which had been collected and verified by PFGE at outbreak investigations of nosocomial infections.

POT method and other gene analysis

Cica Geneous® DNA extraction reagent (Kanto Chemical Co., Ltd., Tokyo, Japan) was used to extract DNA from the colonies for POT method.

Cica Geneous® Staph POT kit: *Staphylococcus aureus* (Kanto Chemical Co., Ltd., Tokyo, Japan) was used for POT analysis for MRSA clinical isolates. The DiversiLab (bioMerieux, France) was used for rep-PCR analysis. All gene analyses were performed in accordance with the manufacturer's instructions.

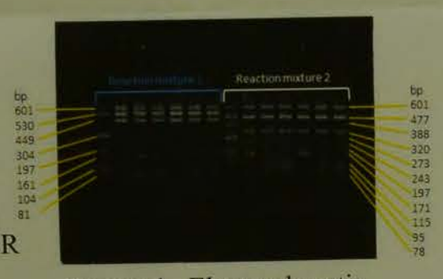


Figure 1. Electrophoretic profile by POT method.

MALDI-TOF MS

The Microflex LT instrument (Bruker Daltonik GmbH, Germany) was used for mass spectrum measurement. Samples were pretreated by ethanol–formic acid extraction, and measured using MALDI Biotyper Version 3.0 (Bruker Daltonik GmbH, Germany). 300 µl of ultrapure water was dispensed into 1.5 ml sample tubes, and 5–10 µg of bacteria from fresh isolated colonies was inoculated and suspended. After mixing with 900 µl of ethanol (Kanto Chemical Co., Ltd., Tokyo, Japan), the suspension was centrifuged for 2 min at 13,000 rpm and the supernatant was removed. After adding 30 µl of 70% formic acid (Kanto Chemical Co., Ltd., Tokyo, Japan) to the precipitation, the suspension was vortexed. 30 µl of acetonitrile (Kanto Chemical Co., Ltd., Tokyo, Japan) was added and mixed; thereafter, the mixture was centrifuged for 2 min at 13,000 rpm and 1 µl of the sample supernatant was used for measurement. Samples were measured in duplicate in the range of 2,000–20,000 m/z with a laser frequency of 60 Hz, and integrated peaks from 240 shots were recorded (6 × 40 laser shots at different locations).

Analysis of mass spectrum data

The FlexAnalysis Version 3.4 was used for comparative analysis of the mass spectra. MALDI Biotyper Version 3.02 and ClinPro Tools Version 2.2 were used for automatic analysis of the mass spectra. Typing was performed based on manually extracted peaks and digitizing cluster analysis was conducted using Ward's method which is one of the methods for multivariable analysis.

For cluster analysis with manually extracted peaks, those without a corresponding peak and those with a corresponding peak were converted to 0 and 1, respectively. This cluster analysis was evaluated based on each spectrum selected by some criteria (Table 1), or all spectra extracted.

Table 1. Criteria of selecting mass spectra

These criteria were determined by 57 MRSA clinical isolates and S. aureus ATCC 29213 data.

1 Reproducible peak extraction
- Signal-to-noise ratio (S/N ratio) is 5.

2. Removal of noise peaks
- Mass range of analysis is 2,000-9,000 m/z.
- Low intensity peaks were excluded.

3. Extraction of MRSA clone-specific peaks

4. Tolerance range
- The tolerable error range was set to ±3 m/z

Result

The results of cluster analysis using selected spectra completely matched the PFGE/POT results (Figure 2).

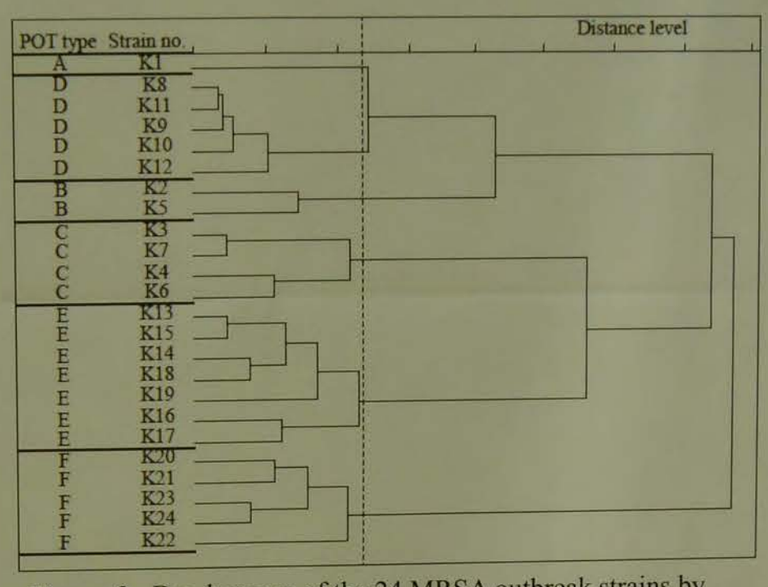


Figure 2. Dendrogram of the 24 MRSA outbreak strains by Ward's method.

The analysis of rep-PCR and MALDI-TOF MS using all spectrum data didn't completely match the PFGE/POT results. The agreement rate of DiversiLab using 95% data was better than 99% data (Table 2).

菌株No	PFGE	POT type	DiversiLab						MALDI Biotyper		
			XJ95%	XJ99%	KL95%	KL99%	PC95%	PC99%	MALDI Biotyper Ver.3.0	All spectrum	select spectrum
K1	A	A	6	14	4	10	4	8	1	1	1
K2	B	B	7	15	5	12	5	9	2	2	2
K5	B	B	7	16	5	12	5	9	5	2	2
K3	C	C	5	12	4	11	4	6	3	3	3
K4	C	C	5	13	4	9	4	7	4	3	3
K6	C	C	5	11	4	11	4	6	4	3	3
K7	C	C	5	1	4	11	4	6	6	4	3
K8	D	D	4	8	3	8	3	5	6	4	4
K9	D	D	4	6	3	8	3	5	1	4	4
K10	D	D	4	10	3	6	3	5	6	4	4
K11	D	D	4	8	3	6	3	5	7	4	4
K12	D	D	7	16	5	12	5	9	2	4	4
K13	E	E	1	4	1	4	1	3	8	5	5
K14	E	E	1	4	1	4	1	3	2	5	5
K15	E	E	1	4	1	1	1	1	2	6	5
K16	E	E	1	3	1	1	1	1	1	6	5
K17	E	E	1	3	1	1	1	1	1	6	5
K18	E	E	1	2	1	3	1	1	8	5	5
K19	E	E	1	1	1	1	1	1	1	7	5
K20	F	F	2	5	2	5	2	4	1	7	6
K21	F	F	3	7	2	2	2	2	1	7	6
K22	F	F	2	5	2	5	2	4	4	7	6
K23	F	F	3	6	2	2	2	2	1	7	6
K24	F	F	3	6	2	2	2	2			

Conclusion

- Cluster analysis using clone-specific peaks is needed when conducting MRSA clonal identification by MALDI-TOF MS.
- The performance of conventional MRSA clonal identification has good capability but it is affected by analytical methodology.
- It is important to realize the performance specification of each measurement system when conducting MRSA clonal identification.

Microbiology

PA-58

Detection for pathogenic bacteria in children in Philippine and Nepal

Chitoshi Sato, Keita Okamura, Shiba Kumar Rai, Pallav VC, Y...

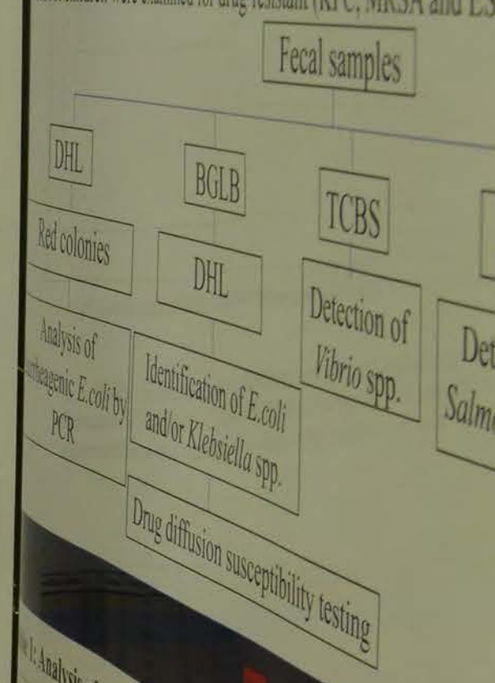
Department of Clinical Laboratory, Okazaki city Hospital
Division of Central Clinical Laboratory, Mie University Hospital
Sojo International College of Science and Technology, & Nat' Inst. of Tropical Health
Institute of Biological Sciences, University of the Philippines Los Banos
School of Health Sciences, Fujita Health University

Purpose

Most of developing country has inferior public health environment. In researchers reported a lot of people are infected with diarrhea disease, death rate of infants and children. The aim of this study to investigate children, in Philippin and Nepal.

Methods

Fecal samples from 7 to 14 years school children were collected in the There were 70 samples of Philippine school children and 150 samples investigation of bacteria in this study was *Vibrio cholera*, *Vibrio parah* *Shigella* spp., *Staphylococcus aureus* and diarrheagenic *Escherichia co* school children were examined for drug-resistant (KPC, MRSA and ES



Results

Table 2: Analysis of diarrheagenic *E.coli* by PCR

Diarrheagenic E.coli	Target gene	Detection numbers (n=70)
EHEC	stx1	0
	stx2	0
EPEC	eae4	0
EIEC	invE	0
EAggEC	aggR	0
ETEC	STp	5
	STh	0
	LT	0
	astA	3
		62

Table 3: Detection of ESBL of *E.coli* with drug diffusion susceptibility testing

No. of samples	CTX	CTX/CVA	CAZ	CAZ/CVA	CPX	CPX/CVA	ESBL
16	0	34	+				
69	14	36	+				
110	15	33	+				
113	14	35	+				
132	0	36	+				
147	11	36	+				

Inhibition zone diameters (mm)

Table 4: Detection of ESBL of *E.coli* with drug diffusion susceptibility testing

No. of samples	CTX	CTX/CVA	CA
16	0	34	
69	14	36	
110	15	33	
113	14	35	22
132	0	36	25
147	11	36	24
	33	36	

No. of samples	me
12	
17	
35	
40	
63	
108	

Discus

Microbiology

PA-58

Detection for pathogenic bacteria in feces of school children in Philippine and Nepal

Chitoshi Sato[1], Keita Okamura[2], Shiba Kumar Rai[3], Paller VG[4], Tadayoshi Hata[5], Seiji Imamura[5]

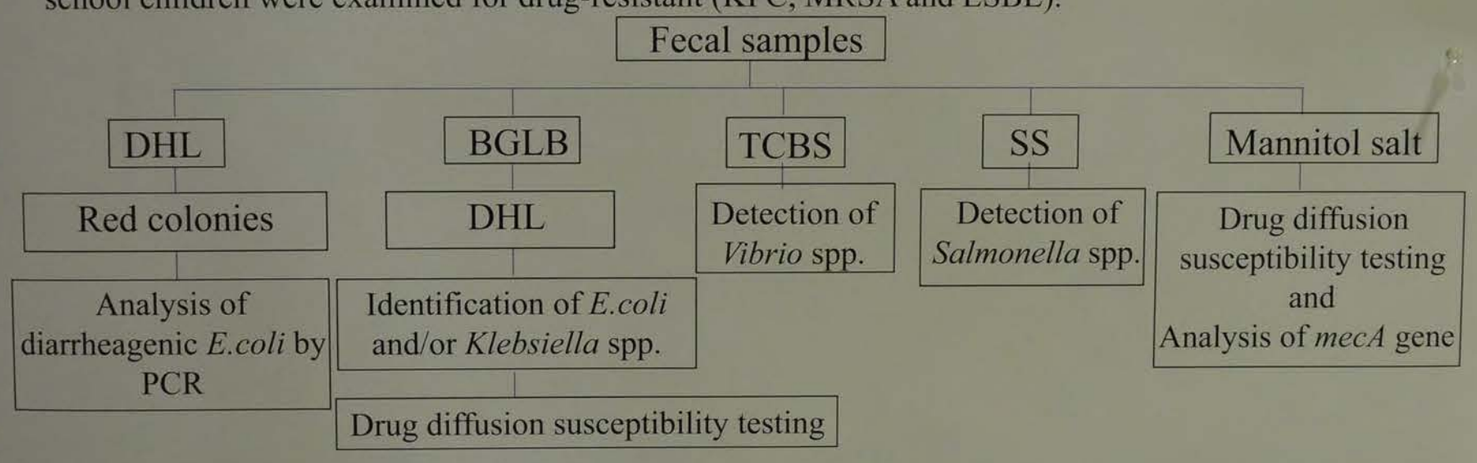
[1] Department of Clinical Laboratory, Okazaki city Hospital
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[3] Shi-Gan International College of Science and Technology & Nat' Inst of Tropical Medicine and Public Health Research
[4] Institute of Biological Sciences, University of the Philippines Los Banos
[5] School of Health Sciences, Fujita Health University

Purpose

Most of developing country has inferior public health environment. In this environment, some researchers reported a lot of people are infected with diarrhea disease, especially enterocolitis cause high death rate of infants and children. The aim of this study to investigate pathogenic bacteria in school children, in Philippin and Nepal.

Methods

Fecal samples from 7 to 14 years school children were collected in these countries and examined in Japan. There were 70 samples of Philippine school children and 150 samples of Nepal school children examined. Investigation of bacteria in this study was *Vibrio cholera*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and diarrheagenic *Escherichia coli.* In addition, feces in Nepal school children were examined for drug-resistant (KPC, MRSA and ESBL).



Results

Table 1: Analysis of diarrheagenic *E.coli* by PCR

Diarrheagenic *E.coli*	Target gene	Detection numbers (n=70)
EHEC	stx1	0
	stx2	0
EPEC	eaeA	0
EIEC	invE	0
EAggEC	aggR	5
ETEC	STp	0
	STh	0
	LT	3
	astA	62

Table 2: Detected as MRSA

No. of samples	*mecA* gene	Cefoxitin disc diffusion testing
12	+	−
17	+	+
35	+	−
40	+	+
63	−	+
108	+	+

Table 3: Detection of ESBL of *E.coli* with drug diffusion susceptibility testing

No. of samples	CTX	CTX/CVA	CAZ	CAZ/CVA	CPX	CPX/CVA	ESBL
1	24	30	25	31	0	34	*
16	15	38	27	33	0	30	*
31	38	37	25	33	30	30	*
70	22	30	15	30	12	30	*
77	15	30	24	30	0	29	*
78	21	30	26	32	0	25	*
101	15	30	25	30	0	30	*
110	15	30	25	33	0	28	*
132	12	30	22	32	0	29	*
138	14	30	26	30	0	27	*

Inhibition zone diameters (mm)

Table 4: Detection of ESBL of *Klebsiella* spp. with drug diffusion susceptibility testing (Kirby-Bauer method)

No. of samples	CTX	CTX/CVA	CAZ	CAZ/CVA	CPX	CPX/CVA	ESBL
16	14	33	22	30	0	28	*
69	15	35	25	30	0	25	*
110	14	30	24	32	0	30	*
113	0	30	13	30	0	25	*
132	11	30	22	30	0	27	*
147	33	30	20	26	30	30	*

Inhibition zone diameters (mm)

Discussion

In developing country, people easily get some antibiotic without medical prescription. Therefore, those system cause drug-resistant bacteria increasing than Japan whose system require medical prescription for getting antibiotic. However, we demonstrated that a carrier rate of ESBL with drug-resistant are not difference between Nepal school children and Japanese. Additionally, KPC- producing bacteria was not detected in this study. This study is contributed to be improvement of public health because those result appear pathogenic bacteria in detail and be international cooperation among Japan, Philippine and Nepal for human health.

Microbiology

PA-59

Seasonal variation of blood stream infection in Japan

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Japanese Red Cross Nagoya Daiichi Hospital
Department of Clinical Laboratory

COI : NONE

Conclusions

- BSI had seasonal variation, which was primarily due to that of GNR-BSI.
- BSI was significantly associated with temperature and humidity.

Background, Material and Methods

Background and Aim

There are seasonal variations in many diseases such as sepsis, stroke, cardiovascular disease, and gastrointestinal disease.

Daniel E. N Engl J Med. 1981
Wroe SJ. Lancet. 1990
Ohmit PA. Clin Chest Med. 2007
Fares A. J Perinatol Med. 2013

Seasonal variation of blood stream infection (BSI) was demonstrated in several countries, however, there were few studies in Japan.

Kato K. Kitr J Clin Microbiol Infect Dis. 2014

Recognition of BSI seasonal trends is significant from perspective of diagnosis, treatment and epidemiologic study.

The aim of this study was to clarify seasonal variation of BSI in Japan.

Material



Japanese Red Cross Nagoya Daiichi Hospital

One of major referral hospitals in Nagoya city with more than 800 beds and 31 clinical departments

30945 blood cultures for 10 years (January 2005 - December 2014)

Only the first blood culture for each patient/episode was included.

Methods

The crude incidence rate (IR) of BSI was calculated per 1000 hospital admissions. The monthly or seasonal variation of IR-BSI was investigated.

Season: Spring (March, April and May)
Summer (June, July and August)
Autumn (September, October and November)
Winter (December, January and February)

The association of IR-BSI with climate (atmospheric pressure, temperature, humidity and weather) of Nagoya city was evaluated.

Classification of bacteria

Gram-negative Rods (GNR)	*Enterococcus* species
Coagulase-negative Staphylococci (CNS)	*Bacillus* species
Staphylococcus aureus	*Candida* species
Streptococcus species	

All cases with positive blood culture were considered to have BSI, even if a blood culture yielded CNS or Bacillus species.

Statistical analyses

Periodicities or cyclical patterns in data was evaluated by Spectral analysis, which contains Fisher's kappa test, Bartlett Kolmogorov-Smirnov test.

Multiple comparison or relationship was evaluated by one-way analysis of variance (ANOVA) or Bonferroni-Dunn method.

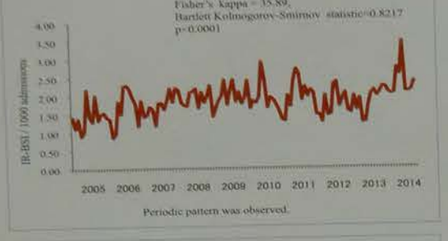
The associations between IR-BSI and climate factors were evaluated by Spearman's rank correlation test.

A p value less than 0.05 was considered statistical significant.

Data analysis was performed using JMP for windows, version10.

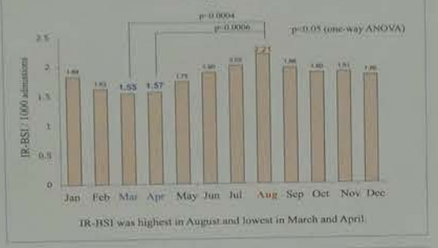
Results (Monthly and Seasonal variation)

Monthly IR-BSI for 10 years



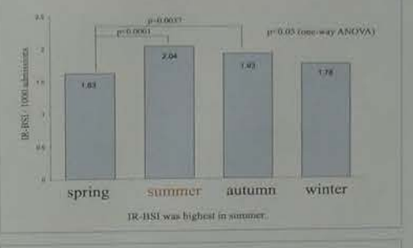
Periodic pattern was observed.

Monthly IR-BSI



IR-BSI was highest in August and lowest in March and April.

Seasonal IR-BSI



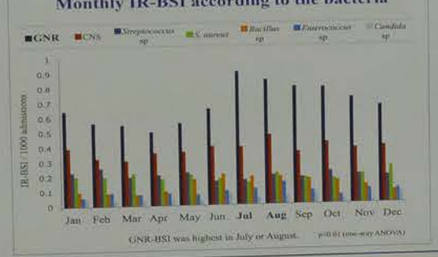
IR-BSI was highest in summer.

IR-BSI for 10-years

	Positive number	IR-BSI (/1000 admissions)	Positive rate of blood culture
All species	**5062**	**1.84**	**16.4%**
Gram negative rod (GNR)	1895	0.69	
Coagulase negative staphylococcus (CNS)	1056	0.38	
Streptococcus species	567	0.21	
S. aureus	544	0.20	
Bacillus species	387	0.14	
Enterococcus species	258	0.09	
Candida species	136	0.05	

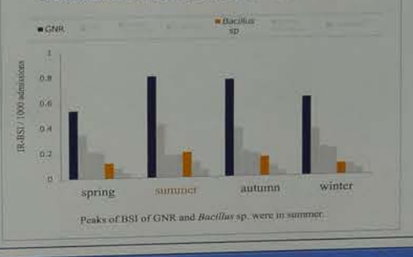
E. coli, *K. pneumoniae*, *P. aeruginosa*, *Enterobacter* species, *H. influenzae* and *P. mirabilis* were predominant organisms among GNR.

Monthly IR-BSI according to the bacteria



GNR-BSI was highest in July or August. p<0.01 (one-way ANOVA)

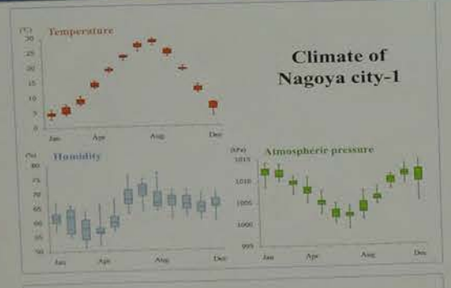
Seasonal IR-BSI according to the bacteria



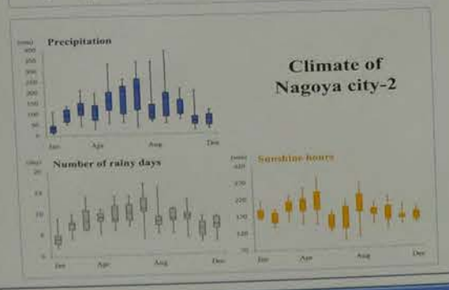
Peaks of BSI of GNR and *Bacillus* sp. were in summer.

Results (Correlation with Climate of Nagoya city)

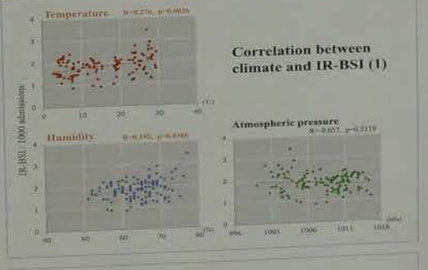
Climate of Nagoya city-1



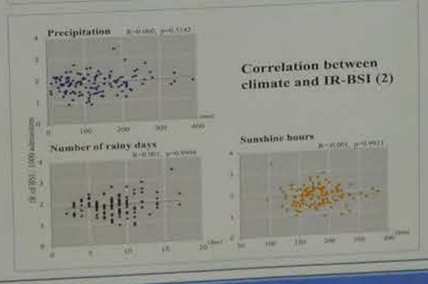
Climate of Nagoya city-2



Correlation between climate and IR-BSI (1)



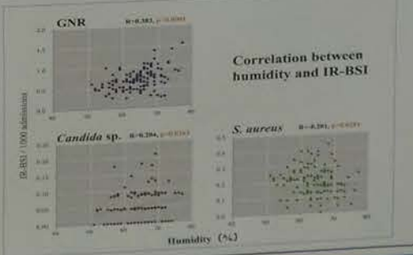
Correlation between climate and IR-BSI (2)



Correlation between temperature and IR-BSI

Bacteria	correlation coefficient	p value
GNR	0.299	**0.0011**
CNS	0.187	**0.0416**
Streptococcus sp	-0.16	0.0803
S. aureus	-0.129	0.1609
***Bacillus* sp**	0.365	**<0.0001**
***Enterococcus* sp**	0.208	**0.023**
Candida sp	0.085	0.3541

Correlation between humidity and IR-BSI



Summary and Discussion

Summary of results

	Monthly variation	Seasonally variation	Correlation with temperature	Correlation with humidity
BSI	↑	↑	positive	positive
GNR	↑	↑	positive	↑
CNS	↑	↑	positive	↑
Streptococcus sp	↑	↑	↑	↑
S. aureus	↑	↑	↑	negative
Bacillus sp	↑	↑	↑	↑
Enterococcus sp	↑	↑	positive	↑
Candida sp	↑	↑	↑	positive

Seasonal variation of BSI in English literature

No	Year	Author	Country	Latitude	Hemisphere	n	Monthly or Seasonal peak	Pathogen
1	2008	Andrews GT	North Carolina, USA; Marseille, France; Melbourne, Australia	35°N to 46°N; 43°N; 37°S	Northern; Northern; Southern	5,288	Summer	*S. pneumoniae*
2	1998	Al-Hasan MN	Dubai, Emirates; Kentucky, USA	25°N; 38°N	Northern; Northern	883	Summer (June-September); Summer (May-October)	*E. coli*
3	2011	Fabich H	Ireland-Dublin based	53°N	Northern	893	Summer (May-October)	*E. coli*
4	2011	Diaz MH	Peru		Southern	111,685	Summer	*S. pneumoniae*; *P. aeruginosa*; *S. enterica* sp; *Enterobacter* sp
5	2001	Al-Hasan MN	Kentucky, USA	38°N	Northern	46	no association	GNR
6	2007	Marra E	Queensland, Australia	23°S	Southern	841	Summer (December-February)	*B. cepacia*
7	2014	Kato K	Kyoto, Japan	35°N	Northern	57	Summer (June-September)	*E. coli*
8	2014	Limmathurotsakul D	Ubon Ratchathani, Thailand	15°N	Northern	79,051	Summer	*B. pseudomallei*
9	2015	Ikegami S	Nagoya, Japan	35°N	Northern	1,827	Summer	GNR; *S. aureus*

Discussion

- BSI was significantly increased in summer, which was primarily due to GNR-BSI, as previous literature reported.
- Common source of GNR-BSI was likely to be urinary tract infection (UTI) by enteric bacteria such as *E. coli* and *K. pneumoniae*.
  - Higher temperature was reported to be associated with *E. coli* growth and virulence, and dehydration-related UTI.
  - Doubling rate of *E. coli* rises with increasing temperature until an optimal growth temperature of 35-36°C.
- BSI-contamination of CNS or Bacillus sp. was reported to have positive correlation with high temperature due to dirty sweat and sebum.
- The reason why BSI was lowest in March and April may be due to low humidity, because dehydration is an important for bacteria inactivation.
- *Staphylococcus aureus* is able to survive in potentially dry environments, such as the human nose, on skin and on inanimate surfaces such as clothing and surfaces.
  (Chaberny-Ivanova P. Arch Microbiol. 2011)
  (Muscholtes I., Microbes Infect. 2011)

Microbiology

PA-60

BACKGROUND

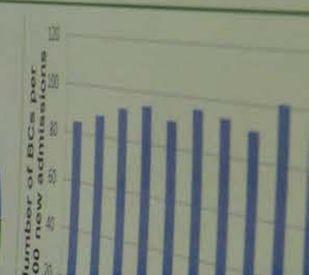
Infection control activities in Japanese hospital has been drastically improved recently by governmental policy changes. In 2010, establishment of the infection control team, comprising several members of professions such as physicians, nurses, pharmacists, and microbiologists to promote hospital infection control activities, has been evaluated by medical service fee. Blood cultures (BCs) are important for diagnosis of bacteremia and the practice pattern of BCs performance including the number of BCs can be a marker of infection control activities. Our objective was to assess infection control activities through BCs results over the past decade in our hospital.

RESULTS

In total, 23,271 BCs in 12,593 cases (11,584 inpatients and 1,009 outpatients) were performed during this study period. As a whole, initial BCs were positive in 11,584 inpatients and in 183 (18.1%) out of 1,009 outpatients, respectively, and in 7.0%) out of 10,678 cases. Possible contamination cases were observed.



Isolated organisms during ten-year period.



Among 2,844 positive BCs, 35% organisms were identified, and top 5 organisms were as follows:
S. aureus (20.9%),
E. faecalis (15.5%),
E. coli (9.9%),
Enterococcus spp.(9.2%),
and *Klebsiella* spp.(6.5%).

There showed a significant increment in annual inpatient incidence of BCs per 1,000 new admissions (coefficient of year: 1.656, 95% CI = 0.696 to 2.617; P = 0.004) and in multiple sets of BCs (coefficient of year: 0.057, 95% CI = 0.043 to 0.071; P < 0.001). There was no significant changes in positive BC rates (coefficient of year: 0.052, 95% CI = -0.100 to 0.204; P = 0.450).

CONCLUSIONS

Multiple sets of BCs performed and their multiple sets have been increased over the past decade. Changes in positive BC rates, in addition, might be changes in methicillin resistance among *S. aureus* decreased over time. Changes in positive BC rates may be related to continuing infection control activities in recent years.

ACKN

We thank to K. Na
Y. Ito, and K. Okan
Laboratory, and A.
Department of Pati
Mie University Hosp

International Federation of Biomedical Laboratory Science
Disclosure of Conflict of Interest
Name of first author : Kazunari Yasuda
I have no COI with regard to our [illegible]

Microbiology

PA-60

Ten-year observation of Blood Culture Results in a Japanese University Hospital

Kazunari Yasuda[1) 2)], Masaki Tanabe[1),2)], Akiko Nakamura[1) 2)], Toru Ogura[3)]
Makoto Morimoto[1)], Kazushi Sugimoto[1)], Kaname Nakatani [1)]

1) Department of Central Laboratory, Mie University Hospital
2) Department of Patient Safety and Infection Control, Mie University Hospital
3) Clinical Research Support Center, Mie University Hospital

BACKGROUND

Infection control activities in Japanese hospital has been drastically improved recently by governmental policy changes. In 2010, establishment of the infection control team, comprising several members of professions such as physicians, nurses, pharmacists, and microbiologists to promote hospital infection control activities, has been evaluated by medical service fee. Blood cultures (BCs) are important for diagnosis of bacteremia and the practice pattern of BCs performance including the number of BCs can be a marker of infection control activities. Our objective was to assess infection control activities through BCs results over the past decade in our hospital.

METHODS

This study was conducted at the Mie University Hospital, a 685-bed educational hospital, in Japan. We performed a retrospective evaluation of the results of all BCs from the both inpatient and outpatient department between January 2006 and December 2015. The blood culture results were evaluated, and statistically analyzed by simple linear regression model. The independent variable for this model was year, and the dependent variables included annual incidence and multiple sets percentage of BCs, positive BCs rates (excluding possible contaminant organisms), and isolated pathogenic organisms. In addition, the trend of the prevalence of methicillin resistance among *Staphylococcus aureus* was also assessed. $P < 0.05$ was considered statistically significant.

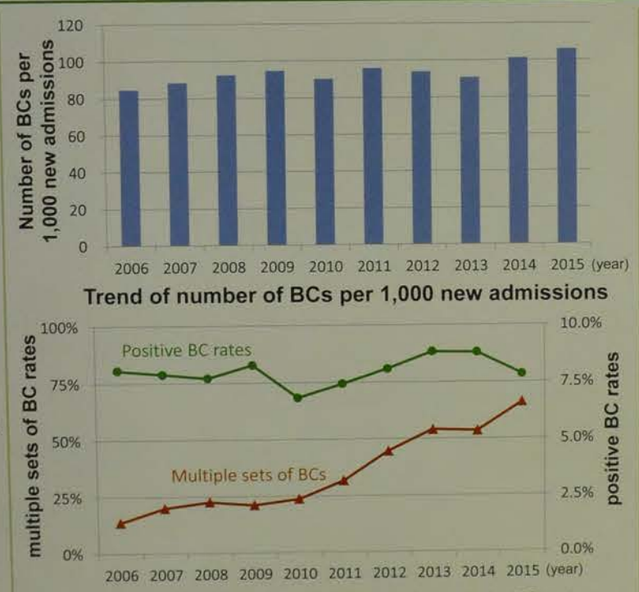
RESULTS

In total, 23,271 BCs in 12,593 cases (11,584 inpatients and 1,009 outpatients) and 10,678 follow-up BC in 4,136 patients were performed during this study period. As a whole, initial BCs were positive in 929 (8.0%) out of 11, 584 inpatients and in 183 (18.1%) out of 1,009 outpatients, respectively. Follow-up BCs were positive in 744 (7.0%) out of 10,678 cases. Possible contamination cases were observed 861 (3.7%) out of 23,271.



Isolated organisms during ten-year period.

Among 2,644 positive BCs, 2,875 organisms were identified, and top 5 organisms were as follows :
CNS(23.8%),
S. aureus(15.5%),
E. coli(9.9%),
Enterococcus spp.(9.2%),
and *Klebsiella* spp.(6.5%).



Trend of number of BCs per 1,000 new admissions

Trend of multiple sets of BCs and positive BC rates

There showed a significant increment in annual inpatient incidence of BCs per 1,000 new admissions (coefficient of year:1.656, 95% CI = 0.696 to 2.617; P = 0.004), and in multiple sets of BCs (coefficient of year: 0.057, 95% CI = 0.043 to 0.071; P < 0.001). There was no significant changes in positive BC rates (coefficient of year: 0.052, 95% CI = -0.100 to 0.204; P = 0.450).

Trends of isolated organisms percentages during Ten-year period.

Dependent variable	β	95% CI	P value
E. coli	0.422	0.071 to 0.774	0.024
K. pneumoniae	0.369	0.022 to 0.716	0.040
E. faecalis	0.349	0.023 to 0.676	0.039
MRSA	-0.925	-1.411 to -0.439	0.002
MSSA	0.408	-0.106 to 0.923	0.105
E. faecium	0.068	-0.240 to 0.377	0.624
Enterobacter spp.	0.216	-0.073 to 0.504	0.124
P. aeruginosa	-0.181	-0.373 to 0.012	0.062
Streptococcus spp.	0.178	-0.262 to 0.618	0.378
Candida spp.	-0.447	-0.985 to 0.091	0.091
CNS	-0.341	-1.146 to 0.464	0.358
Bacillus spp.	0.047	-0.294 to 0.387	0.761

β: coefficient of year



The prevalence of methicillin resistance among *S. aureus* isolates

Isolation frequencies of *E. coli*, *K. pneumoniae* and *E. faecalis* were increased during ten-year period, whereas isolation of MRSA was decreased. The prevalence of methicillin resistance among *S. aureus* isolates decreased from 75.0% in 2006 to 31.0% in 2015.

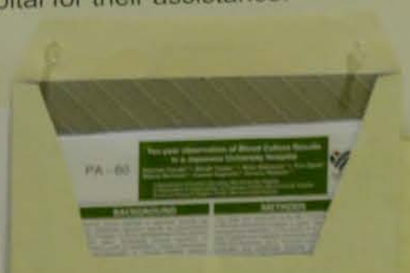
CONCLUSIONS

Frequencies of BCs performed and their multiple sets percentages have been increased over the past decade without change in positive BC rates, in addition, percentage of methicillin resistance among *Staphylococcus* decreased over time.
These favorable trends may be related to continuing infection control activities in recent years.

ACKNOWLEGEMENT

We thank to K. Nakazawa, E. Tomatsu, M. Wakabayashi, Y. Ito, and K. Okamura of the Department of Central Laboratory, and A. Arai and D. Yamasaki of the Department of Patient Safety and Infection Control in the Mie University Hospital for their assistance.

International Federation of Biomedical Laboratory Science
Disclosure of Conflict of Interest
Name of first author : Kazunari Yasuda
I have no COI
with regard to our presentation.



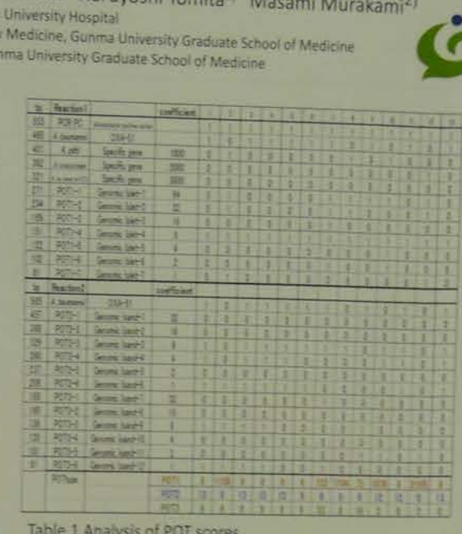


Table.1 Analysis of POT scores



Fig.3 pulsed-field electrophoresis, Lane M is marker.

【Conclusion】
As for the pulsed-field electrophoresis, the operation is complicated, and time and a special device are necessary. Software is necessary for analysis.
With the POT method, it took only four hours for us to examine the bacteria from DNA extraction to the identification of the bands.
The preparation of the reagents are easy, and specific equipment are not necessary for POT method. Therefore, POT method is possibly used in many clinical laboratories, and is a very useful tool to analyze strains for suspected nosocomial infections in clinical settings.

Microbiology

PA-61

Dissemination of extended-spectrum β-lactamase producing *Escherichia coli* in Indonesia

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Introduction

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* is one of the health problems worldwide. The ESBL-encoding genes are known as bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, and the spread of $bla_{CTX-M-15}$ cause great concern. Additionally, it has been reported the global spread of the ESBL producing *E. coli* belonged to phenogenetic group B2-sequence type (ST) 131 or A-ST617 by multilocus sequence typing (MLST). The STs are grouped into clonal complexes by the allelic similarities and therefore ST617 is belong to clonal complex (CC) 10 containing several STs (ST10, ST44 and ST167 etc.) In Indonesia, bacterial diarrhea caused by diarreagenic *E. coli* (DEC) is one of the most common causes of mortality among children. There are few reports about antimicrobials resistant DEC in Indonesia. The present study was aimed to investigate the antimicrobial susceptibility and molecular characteristics of clinical *E. coli* isolated from pediatric diarrhea patients in Indonesia.

Materials and Methods

DEC strains: A total of 124 *E. coli* strains were isolated from pediatric diarrhea patients in Soetomo Academic Hospital, Surabaya, Indonesia between January 2012 and December 2012. **Antimicrobial susceptibility test:** All isolates were tested 15 antimicrobial susceptibilities with the Clinical and Laboratory Standards Institute (CLSI) confirmatory test as follows: cefotaxime (CTX), ceftazidime (CAZ), cefpodoxime (CPDX), cefepime(CFPM), imipenem (IPM), aztreonam (AZT), piperacillin (PIPC), ampicillin (ABPC), Sulbactam/Ampicillin (S/A), gentamicin (GM), amikacin (AMK), tetracycline (TC), nalidixic acid (NA), ciprofloxacin (CPFX), trimethoprim-sulfamethoxazole (STX). **ESBL screening test:** ESBL production was initially tested using both CTX and CAZ disks alone and in combination with clavulanic acid (CVA) by double disk synergy test. Quality control was performed with *E.coli* ATCC 25922. **PCR and sequencing:** We detected the presence of bla_{CTX-M}, bla_{TEM}, bla_{SHV}, DEC-encoding genes (*eaeA, stx1, stx2, est, elt, aggR* and *invE*), the four main *E. coli* phylogenetic groups (A, B1, B2, D) and plasmid replicon (FIA, FIB, FIC, FII, F_{repB}, HI1, HI2, I1, L/M, N, P, W, T, A/C, K, B/O, X and Y) by PCR and sequencing. DEC categories were decided according to the instruction of Cica Geneus Pathogenesis Gene Detection PCR Kit (KANTO KAGAKU Inc.). ESBL-positive isolates were analyzed for phylogenetic relationships by using MLST and rep-PCR. **Statistical analysis:** Fisher's exact tests were used to evaluate relationships between variables.

Results

Antimicrobial susceptibility test: ESBL-producing *E. coli* were detected in 25 (20.2%) of 124 strains. ESBL-producing strains had significant resistance to AZT**, PIPC**, GM**, NA** and CPFX** more than non ESBL-producing strains (**: $p<0.01$) (Fig. 1). All 124 strains were susceptible to IPM. **ESBL genes:** Among 25 ESBL-producing strains, 21 (84.0%) were positive for $bla_{CTX-M-15}$, containing 6 (24.0%) bla_{TEM-1}-producing strains. One (4.0%) was $bla_{CTX-M-15}$ and bla_{SHV-7}, 2 (8.0%) were $bla_{CTX-M-14}$ and bla_{TEM-1}, 1 (4.0%) was $bla_{CTX-M-1}$ and 1 (4.0%) was bla_{TEM-1}. **DEC:** Among 124 strains, 26 (21.0%) strains including 2 (1.6%) ESBL-producing strains had DEC-encoding genes (Table 1). **Phylogenetic groups:** ESBL-positive strains belonged to *E. coli* phylogenetic group A was 12 (48.0%), B1 was 3 (12.0%), B2 was 2 (8.0%), D was 8 (32.0%) strains. **Plasmid replicon typing:** In ESBL-positive stains, high frequency types of plasmid replicon were IncFIA, IncI1 ($p<0.01$), and IncHI2 ($p<0.05$), but not detected HI2, L/M, W, T, X and FII (Fig. 2). **MLST:** Among 25 ESBL-producing strains, 9 (36.0%) strains belonged to CC10 (ST10, ST44, ST167, and ST617) having CTX-M-15 and resistance to CPFX by MLST (Fig. 3). **rep-PCR:** The most common rep-PCR cluster (>95%) accounted 5 (20%) which were CTX-M-15-producing *E. coli* A-CC10, out of the 25 ESBL-positive strains (Fig. 4).

Table 1. The relationship of DEC-encoding genes and ESBL types.

DEC	No. of isolates	ESBL type	*eaeA*	*stx1*	*stx2*	*est*	*elt*	*aggR*	*invE*
EAEC	1	CTX-M-14 TEM-1	−	−	−	−	−	+	−
	14	−	−	−	−	−	−	+	−
EHEC	1	−	+	+	+	−	−	−	−
	4	−	−	+	−	−	−	−	−
ETEC	2	−	−	−	−	+	−	−	−
	1	−	−	−	−	+	+	−	−
EPEC	1	TEM-1	+	−	−	−	−	−	−
	1	−	+	−	−	−	−	−	−
EIEC	1	−	−	−	−	−	−	−	+

*DEC categories were EAEC (*aggR*+), EHEC (*stx1*+ or *stx2*+), ETEC (*est*+ or *elt*+), EPEC (*eaeA*+) and EIEC (*invE*+). +: detected, −: not detected

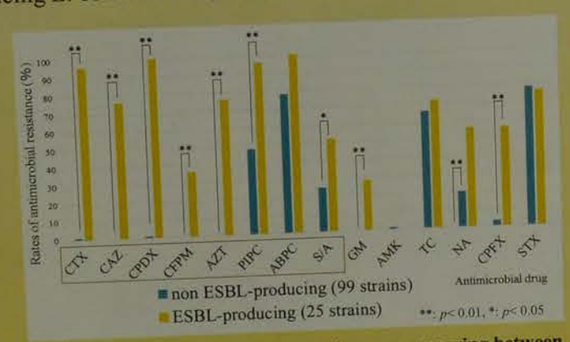


Figure 1. Rates of antimicrobial resistance comparing between ESBL-producing strains and non ESBL-producing strains.

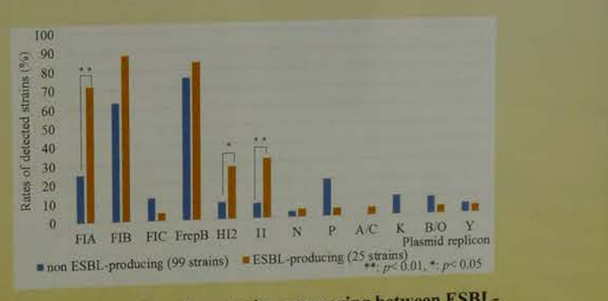


Figure 2. Plasmid replicon typing comparing between ESBL-producing *E. coli* and non ESBL-producing *E. coli*.

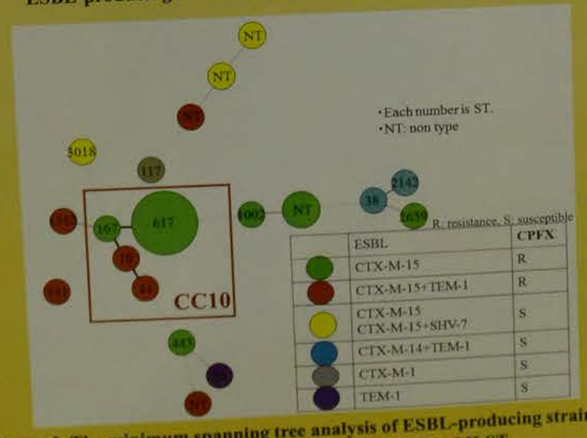


Figure 3. The minimum spanning tree analysis of ESBL-producing strains based on ESBL types and the susceptibility to CPFX by MLST.

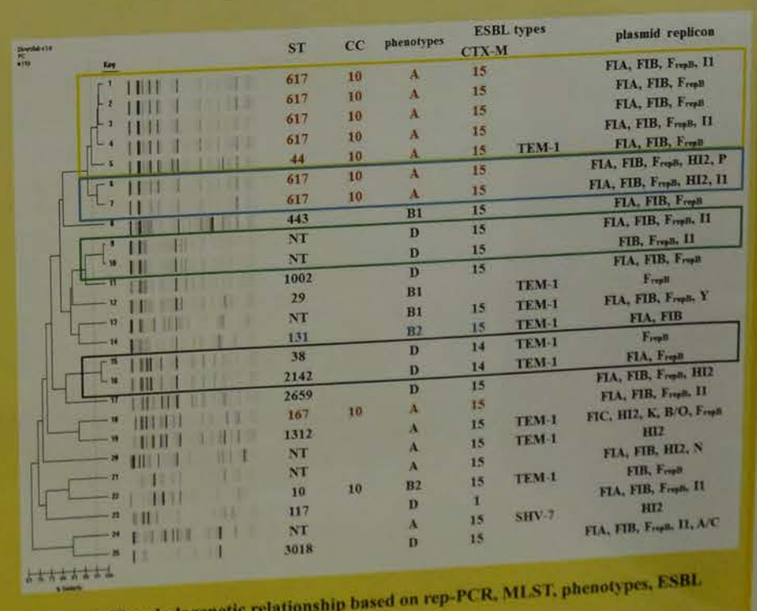


Figure 4. The phylogenetic relationship based on rep-PCR, MLST, phenotypes, ESBL types and plasmid replicon types.

Conclusion

ESBL-producing *E. coli* A-CC10 having CTX-M-15 were isolated from pediatric diarrhea patients in Indonesia, and had higher antibiotic resistance. At this point, IPM and AMK still showed good susceptibilities even in ESBL-producing *E. coli* A-CC10 strains.

Microbiology

PA-62

Epidemiology analysis of the Acinetobacter by the PCR-based Phage Open Reading Frame Typing method

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【Background】
The *Acinetobacter* spp. is a gram-negative bacillus of the glucose non-fermentation. *Acinetobacter baumannii* (*A.baumannii*) is one of the causative organisms of hospital infection. It is necessary to confirm the presence or absence of blaoxa-51-like that *A.baumannii* contains on its chromosome, and 16S rRNA genetic analysis for accurate identification of the strain. Therefore it is difficult to identify *A.baumannii* in the routine examination. Recently PCR-based Phage Open Reading Frame Typing (POT) method has been introduced to clinical laboratory. POT enables us to identify several kinds of bacteria including *A.baumannii*, and to analyze them by examination among the different strains.

【Methods】
We studied for 13 strains of *Acinetobacter* genus bacteria isolated in our hospital from January to December in 2014. In addition to the POT method, we performed drug sensitivity testing and pulsed-field gel electrophoresis(PFGE). We performed POT analysis and pulsed-field electrophoresis according to the manufactures instructions (Fig.1).

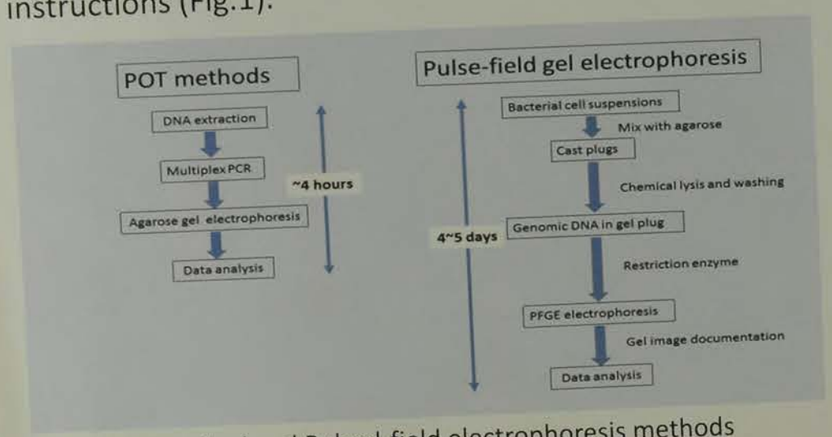


Fig.1 POT method and Pulsed-field electrophoresis methods

【Results】
Thirteen strains were classified into eight POT types by the POT method(Fig.2&Table1). Eight of 13 strains were identified with *A.baumannii.* POT1 is associated with an ST type of the MLST method. We were able to identify International clone II (IC II)in one strain(No.7).Results of PFGE showed Figure 3.



Fig.2 Agarose gel electrophoresis patterns of POT methods, Lane L is ladder marker, Lane PC is positive control

bp	Reaction1		coefficient	1	2	3	4	5	6	7	8	9	10	11	12	13
553	PCR PC	*Acinetobacter* positive control		1	1	1	1	1	1	1	1	1	1	1	1	1
465	*A.baumannii*	OXA-51		1	0	1	1	1	1	1	0	1	0	1	0	1
401	*A.pitii*	Specific gene	1000	0	1	0	0	0	0	0	1	0	1	0	0	0
362	*A.nosocomialis*	Specific gene	2000	0	0	0	0	0	0	0	0	0	0	0	1	0
321	*A.sp.* strain 13TU	Specific gene	3000	0	0	0	0	0	0	0	0	0	0	0	0	0
271	POT1-1	Genomic Islet-1	64	0	1	0	0	0	0	1	1	1	1	0	1	0
234	POT1-2	Genomic Islet-2	32	0	1	0	0	0	0	1	1	0	0	0	1	0
189	POT1-3	Genomic Islet-3	16	0	0	0	0	0	0	1	0	0	0	0	0	0
151	POT1-4	Genomic Islet-4	8	1	1	1	1	1	1	1	1	1	1	1	1	1
122	POT1-5	Genomic Islet-5	4	0	0	0	0	0	0	0	0	0	1	0	0	0
102	POT1-6	Genomic Islet-6	2	0	0	0	0	0	0	1	0	0	1	0	0	0
81	POT1-7	Genomic Islet-7	1	0	1	0	0	0	0	0	0	0	0	0	1	0
bp	Reaction2		coefficient													
565	*A.baumannii*	OXA-51		1	0	1	1	1	1	1	0	1	0	1	0	1
457	POT2-1	Genomic Isand-1	32	0	0	0	0	0	0	0	0	0	0	0	0	0
388	POT2-2	Genomic Isand-2	16	0	0	0	0	0	0	0	0	0	0	0	0	0
329	POT2-3	Genomic Isand-3	8	1	0	1	1	1	1	1	0	1	1	0	1	
280	POT2-4	Genomic Isand-4	4	1	0	1	1	1	0	0	0	1	1	0	1	
237	POT2-5	Genomic Isand-5	2	0	0	0	0	0	0	0	0	0	0	0	0	
209	POT2-6	Genomic Isand-6	1	1	1	1	1	1	1	0	0	0	1	0	1	
185	POT3-1	Genomic Isand-7	32	0	0	0	0	0	0	1	0	0	0	0	0	
160	POT3-2	Genomic Isand-8	16	0	0	0	0	0	0	0	0	0	0	0	0	
139	POT3-3	Genomic Isand-9	8	1	1	1	1	0	0	0	0	1	0	0	0	
120	POT3-4	Genomic Isand-10	4	0	0	0	0	0	0	0	0	0	0	0	0	
101	POT3-5	Genomic Isand-11	2	0	0	0	0	0	0	0	0	1	1	0	1	
81	POT3-6	Genomic Isand-12	1	1	1	1	1	0	0	1	0	0	0	0	0	
	POTtype		POT1	8	1105	8	8	8	8	122	1104	72	1078	8	2105	8
			POT2	13	0	13	13	13	9	8	8	0	12	13	0	13
			POT3	9	9	9	9	0	0	33	0	10	2	0	2	0

Table.1 Analysis of POT scores



Fig.3 pulsed-field electrophoresis, Lane M is marker.

【Conclusion】
As for the pulsed-field electrophoresis, the operation is complicated, and time and a special device are necessary. Software is necessary for analysis.
With the POT method, it took only four hours for us to examine the bacteria from DNA extraction to the identification of the bands.
The preparation of the reagents are easy, and specific equipment are not necessary for POT method. Therefore, POT method is possibly used in many clinical laboratories, and is a very useful tool to analyze strains for suspected nosocomial infections in clinical settings.

Microbiology

PA-63

Comparison of phage open reading flame typing and rep-PCR method for detection of a multi-drug resistant *Pseudomonas aeruginosa* strains

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Introduction

Although *Pseudomonas aeruginosas* usually rarely cause infection in healthy people, it is important as bacteria causing healthcare-acquired infections, such as opportunistic infections for the compromised host in hospitalized patients. Among the *P. aeruginosa*, the multi-drug resistant *P. aeruginosa* (MDRP) infections increase mortality, morbidity, and hospital costs. We conducted a comparative study of epidemiological analysis in MDRP by phage open reading flame typing (POT) and rep-PCR method for detection of a MDRP in monitoring nosocomial infection.

Subjects and Methods

The subject strains as MDRP(47 strains) used in this study were obtained from the stored strains in Kitasato University Research Center for infectious and antimicrobials, and those in Saga University Hospital. POT method was conducted by a multiplex PCR using a Cica Genius Pseudo POT kit ® (Kanto Chemical Co., Inc.), following assessment and analyses of DNA band patterns (Table 1, Figure 1). The rep-PCR method was carried out using a DiversiLab® fingerprinting kit (Sysmex bioMérieux), and the obtained PCR products were analyzed by DiversiLab System (Figure 2). Over 95% similarity by fingerprinting patterns of PCR products was diagnosed as similar strains.

Table 1 Types of open reading flame (ORF) (target area) and product size of PCR

	POT Number	Amplification size(bp)	Target area
Reaction mixture 1	PCR Positive control	506	*P. aeruginosa* Positive control
	POT1-1	336	Islet-1
	POT1-2	281	Islet-2
	POT1-3	235	Islet-3
	POT1-4	201	Islet-4
	POT1-5	175	Islet-5
	POT2-1	151	*vim*
	POT2-2	126	Prophage-1
	POT2-3	103	Prophage-2
	POT2-4	85	Prophage-3
Reaction mixture 2	PCR Positive control	506	*P. aeruginosa* Positive control
	POT1-6	324	Islet-6
	POT1-7	271	Islet-7
	POT1-8	238	Islet-8
	POT1-9	204	Islet-9
	POT1-10	176	Islet-10
	POT2-5	150	Prophage-4
	POT2-6	124	Prophage-5
	POT2-7	105	imp

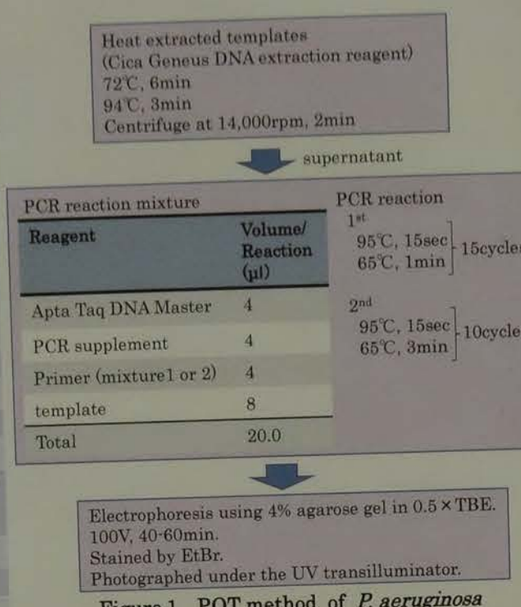


Figure 1 POT method of *P. aeruginosa*

The major steps generating rep-PCR DNA fingerprints are as follows:
- Extract DNA from purified microbial cultures.
- Log sample information into the Diversilab ® software.
- Perform rep-PCR amplification in a thermal cycler.
- Separate the DNA fragments using the Agilent 2100 Bioanalyzer.
- Compare fingerprints using the statistical analysis feature of the Diversilab ® software.

Reagent	Volume/ Reaction (μl)
Rep-PCR MM1	18.0
GeneAmp 10× PCR Buffer	2.5
Primer Mix O	2.0
AmpliTaq DNA Polymerase	0.5
Total	23.0

rep-PCR amplification			
Initial denaturation	94℃	2min	
Denaturation	94℃	30sec	Repeat cycle
Annealing	50℃	30sec	35cycles
Extension	70℃	90sec	
Final extension	70℃	3min	
Hold	4℃	∞	

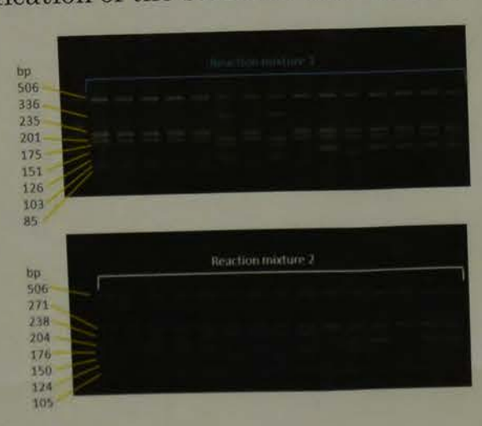
Figure 2 rep-PCR method of *P. aeruginosa*

Table 2 Characteristics of each method for epidemiological studies

Methods	PFGE	Rep PCR (DiversiLab)	POT
Targets	Whole genome	Repetitive sequence	22 coding regions
principles		PCR	multiplex PCR
simplicity	△	◎	◎
Test time	3～5 days	4hours	4hours
Target microorganisms	Various	28 kit	MRSA *P. aeruginosa*
Total costs (including devices)	400～600 million yen	≒1000 million yen	150 million yen
cost/1 sample	700 yen	1500 yen	1000 yen

Results

The 47 strains were classified into 12 types by POT method and 15 types by rep-PCR method, respectively (Figure 3,4). POT number 207-25 were the most common (18 strains) in POT method, following number 207-24 (8 strains), number 202-72 (7 strains), and number 205-25 (3 strains), respectively. According to a cutoff value as 95 % in rep-PCR method, 18 strains of POT number 207-25 were further separated into 6 types, 8 strains of number 207-24 were separated into 4 types and 3 strains of number 205-25 were separated into 3 types, respectively. However, when the cut-off value of rep-PCR method is used as 85 % or more, the classification of the starains were in good agreement with those by POT method .



POT Number	POT Type	Strain
207-25	○	18
207-24	●	8
200-72	△	2
202-72	▲	7
205-25	◇	3
620-52	□	2
620-54	■	2
636-54	◆	1
636-52	★	1
203-41	☆	1
104-0	▽	1
805-0	▼	1

Figure 3 POT method result of *P. aeruginosa*

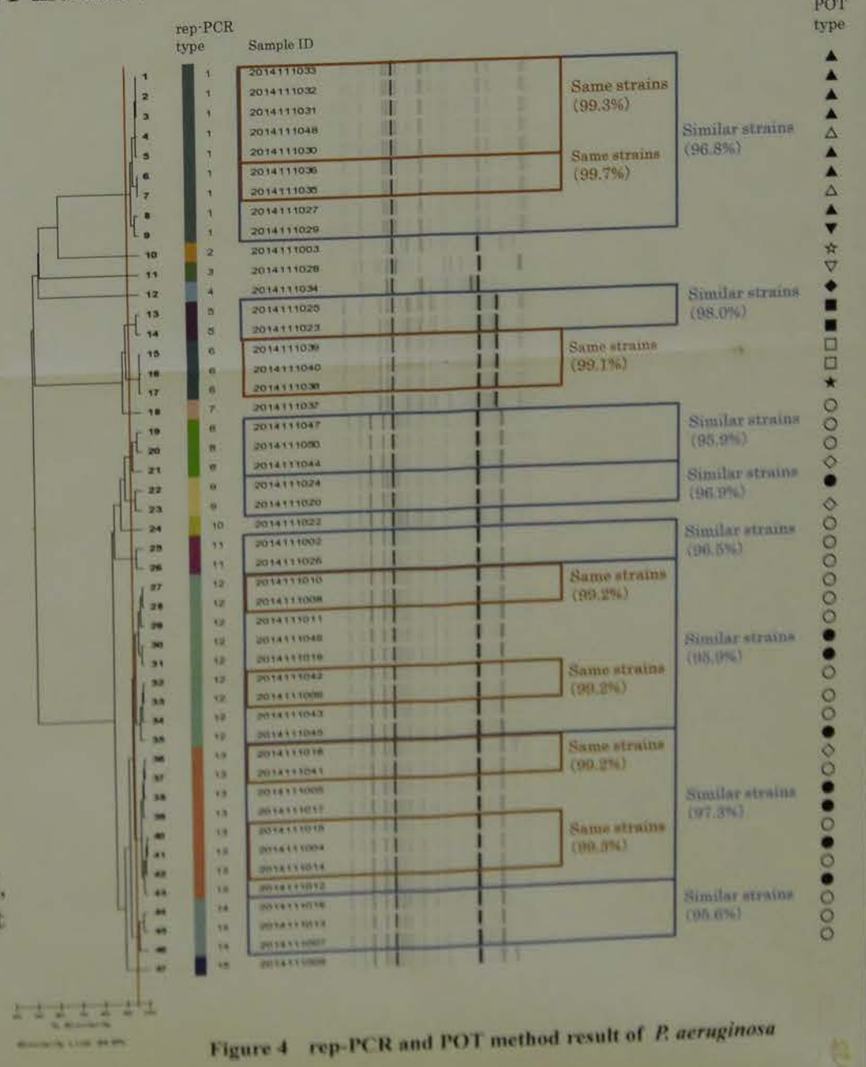


Figure 4 rep-PCR and POT method result of *P. aeruginosa*

Discussion

According to the difference of target sequences for amplification and classification methods using obtained genotypes, epidemiological classification for MDRP by POT and rep-PCR method did not show completely match for the determination of the cluster of strains. However, when the cut-off value of rep-PCR method is decides as 85 % or more, the results of epidemiological classification were in good agreement with those of POT method. Although PFGE is a standard method for molecular epidemiological study of microorganisms, it is expensive, time consuming, and requires specific technique to obtain stable results. In comparison with PFGE, POT and rep-PCR methods are simple and easy by using a kit, and it takes about only 4 hours for an assay (Table2). Furthermore, it is also possible the comparison with the previous data. All results indicate that both methods are useful for applying nosocomial infection control.

Microbiology

PA-64

Antimicrobial resistance pattern of *Campylobacter coli* isolated from diarrheal patients and raw meat samples

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Introduction

Campylobacter is widely known as one of the most common food borne pathogens and *C. coli* is a predominant causative agent of Campylobacteriosis. In recent years, several studies have reported the increasing levels of antimicrobial resistance in *C. coli*. The resistant strains have been isolated from patients with diarrhea, who were not exposed to any antimicrobials, and it is thought that the antimicrobial resistant strains may have originated from domestic animals. In this study, a comparison between fecal and raw meat originated *Campylobacter coli* was made, for better understanding of the present resistance pattern of this organism isolated from local sources.

Materials and Methods

Fecal samples from 100 diarrheal patients and 131 raw meat samples (poultry: 66, swine: 42, and bovine: 23) were collected from retail stores located in Hirosaki city, Japan. *C. coli* was isolated by the rinsed sample culture method and identified through multiplex PCR (mPCR). Antimicrobial resistance pattern was tested using the disk diffusion assay using erythromycin (EM), tetracycline (TC), ciprofloxacin (CPFX), ofloxacin (OFLX), and fosfomycin (FOM).

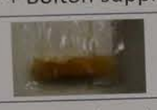
1. The rinsed sample culture method

Meat samples 25 g + Bolton broth 100 mL (→ Sample broth)



↓ homogenize 1 min

Sample broth 10 mL + Defibrinated horse blood 500 µL + Bolton supplement 100 µL (→ Sample culture)



↓ capneic incubation at 42±1°C, 24h

Sample culture 20 µL + Skirrow/Butzler agar

↓ capneic incubation at 37±1°C, 48h



2. Primers and mPCR amplification

Sequence of oligonucleotide primers of multiplex PCR used in this study

Species	Sequence (5' to 3')	Size (bp)	Reference
C. jejuni	CAAATAAAGTTAGAGGTAGAATGT CCATAAGCACTAGCTAGCTGAT	161	Wang
C. coli	GGTATGATTTCTACAAAGCGAG ATAAAAGACTATCGTCGCGTG	502	Linton
C. lari	TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC	251	Wang

General reaction mixture for PCR

10X Ex Taq™ Buffer	2.5 µL
dNTP Mixture (2.5 mM each)	2 µL
TaKaRa Ex Taq™ (5 units/ µL)	0.125 µL
Primer-F (25 µM)	each 0.25 µL
Primer-R (25 µM)	each 0.25 µL
DNA template	2.5 µL
Distilled water	up to 25 µL

PCR condition

94°C 1 min	preheating	
94°C 1 min	denaturation	25 cycles
55°C 1 min	annealing	
72°C 1 min	extension	
72°C 10 min	final extension	

3. The disk diffusion assay (Kirby-Bauer method)

Using growth colony onto the plate, ensure a turbidity of 0.5 McFarland



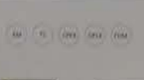
↓

Inoculate Mueller-Hinton(MH) agar with 5% Horse blood by swabbing (3 times)



↓

Place each paper disks on the MH agar



↓ capneic incubation at 37±1°C, 18h



	R (mm)	I (mm)	S (mm)
EM	6	–	–
TC	11	12–14	15
CPFX	6	–	–
OFLX	12	13–15	16
FOM	10	11–15	16

R: resistant
I: intermediate
S: suspectible

Results

A total of 24 *C. coli* (10 from fecal and 14 from raw meat samples) were isolated and the results of the disk diffusion assays revealed that the isolates from feces and raw meat samples had the following respective values of resistance to antibiotics: EM (30.0%, 21.4%), TC (70.0%, 57.1%), CPFX (30.0%, 28.6%), OFLX (30.0%, 28.6%), and FOM (0%, 0%).

1) mPCR

	C. jejuni	*C. coli*	*C. jejuni* + *C. coli*	*C. coli* + *C. lari*
pourty	17			
swine		7	2	1※
bovine		1	1	

※Not examined

2) The disk diffusion assay

1) Feces from diarrheal patients

	EM	TC	CPFX	OFLX	FOM
1	R	R	R	R	S
2	R	R	R	R	S
3	S	R	R	R	S
4	R	R	S	S	S
5	S	R	S	S	S
6	S	R	S	S	S
7	S	R	S	S	S
8	S	S	S	S	S
9	S	S	S	S	S
10	S	S	S	S	S

2) Raw meat samples

	EM	TC	CPFX	OFLX	FOM
1※	S	S	S	S	S
2	S	S	R	R	S
3※	S	R	R	R	S
4	R	S	S	S	S
5	R	R	R	R	S
6	S	R	S	S	S
7	S	R	S	S	S
8	R	R	S	S	S
9	S	I	S	S	S
10※	S	R	S	S	S
11	S	R	R	R	S
12	S	S	S	S	S
13	S	S	S	S	S
14	S	R	S	S	S

※ Different colony of same sample

Discussion

FOM-resistance was not observed in any of the *C. coli* isolates. On the other hand, TC-resistance was found to be high (57.1-70.0 %), particularly, in strains of fecal origin (70.0 %). Overall, the resistance pattern of *C. coli* was found to vary for each antimicrobial and no relationship was observed between the antimicrobial resistance of *C. coli* isolated from fecal samples and that from raw meat samples.

Acknowledgment and COI

We thank S. Tsukiashi, Clinical Inspection Center, Hirosaki Medical Association, for supplying the *Campylobacter* strains.

The authors have no conflict of interest (COI) directly relevant to the content of this article.

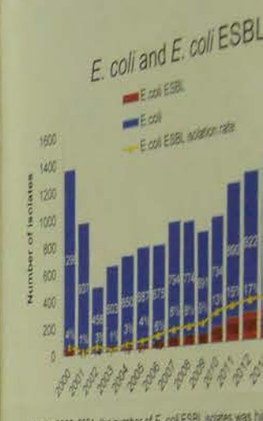
Microbiology

PA-65

Extended-spectrum beta-lactam
control worldwide. Furthermore,
at our hospital.

For the pathogens were Esche
2000 to 2015. ESBL strains wer
Aggregation method:
One patient/year.
Isolation and drug resistance ra
We determined the ratio of each
cultures.

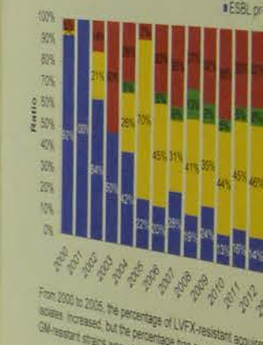
E. coli and E. coli ESBL



During 2006–2001, the number of E. coli ESBL isolates was hi
been because drug susceptibility tests were conducted on eve
collected from stool samples during these years.

Drug resis

E. coli ESBL

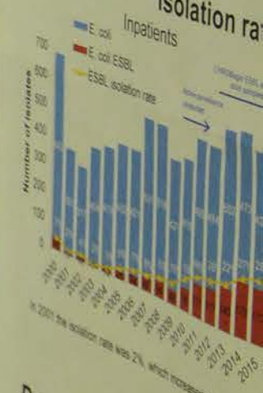


From 2000 to 2005, the percentage of LVFX-resistant acqui
isolates increased, but the percentage has remained stable
GM-resistant strains were also resistant to LVFX.

*E. coli ESBL isolates increased compared to

Isolation ra

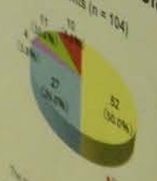
Inpatients



In 2001 the isolation rate was 2%, which increased to 28% in 20

Proportion of each E. coli E
specimen isolated in 201

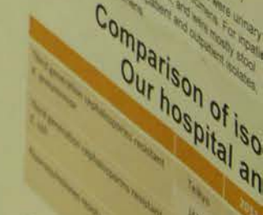
Outpatients (n = 104)



Inpatients (n = 21



Comparison of iso
Our hospital an



- Isolation rates of ESBL-producing bacte
- The resistance to antibiotics by E. coli E
susceptibility tests and confirming drug e
- The high isolation rates of ESBL at our h
- The detection rates in outpatients were h
- Continuous observation for drug-resistan

Microbiology

PA-65

Isolation and drug susceptibility trends of extended spectrum beta-lactamase (ESBL)-producing bacteria at Teikyo University Hospital

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1)Department of Central Clinical Laboratory, Teikyo University School of Medicine

2)Department of Microbiology and Immunology, Teikyo University School of Medicine

Purpose

Extended-spectrum beta-lactamase (ESBL)-producing bacteria continue to be a major problem for healthcare-associated infection control worldwide. Furthermore, the bacteria vary in drug susceptibility. Therefore, we investigated trends in ESBL-producing isolates at our hospital.

Subjects and Methods

For the pathogens were *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, drug susceptibility tests were conducted from 2000 to 2015. ESBL strains were confirmed using the double disk synergy test (DDST).

Aggregation method:

One patient/year.

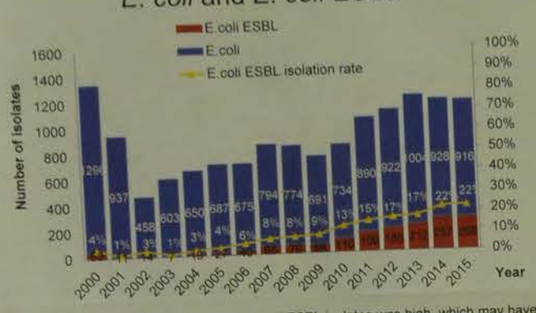
Isolation and drug resistance rates of each bacterium from 2000–2015.

We determined the ratio of each specimen to the total *E. coli* ESBL isolates in 2015. Detailed analysis was conducted using blood cultures.

Results

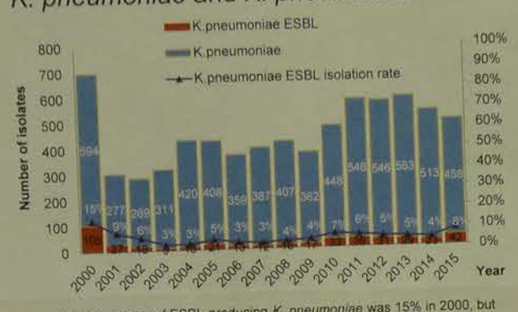
Transition of isolates

E. coli and *E. coli* ESBL



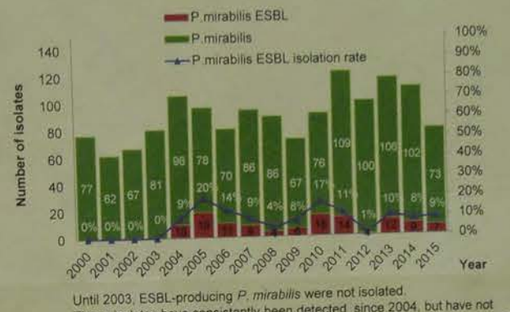
During 2000–2001, the number of *E. coli* ESBL isolates was high, which may have been because drug susceptibility tests were conducted on every *E. coli* isolate collected from stool samples during these years.

K. pneumoniae and *K. pneumoniae* ESBL



The percentage of ESBL-producing *K. pneumoniae* was 15% in 2000, but was 3–9% in 2001–2015.

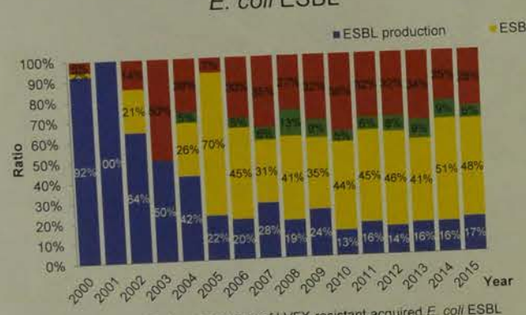
P. mirabilis and *P. mirabilis* ESBL



Until 2003, ESBL-producing *P. mirabilis* were not isolated.
These isolates have consistently been detected since 2004, but have not increased in number.

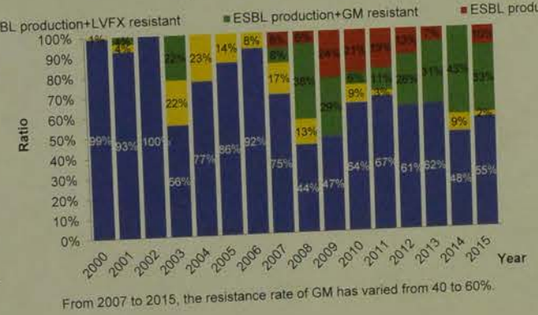
Drug resistance rate to levofloxacin (LVFX) and gentamicin (GM)

E. coli ESBL



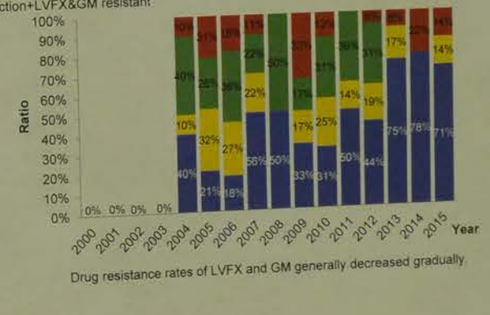
From 2000 to 2005, the percentage of LVFX-resistant acquired *E. coli* ESBL isolates increased, but the percentage has remained stable since then. Most GM-resistant strains were also resistant to LVFX.

K. pneumoniae ESBL



From 2007 to 2015, the resistance rate of GM has varied from 40 to 60%.

P. mirabilis ESBL



Drug resistance rates of LVFX and GM generally decreased gradually.

E. coli ESBL sensitivity in 2015

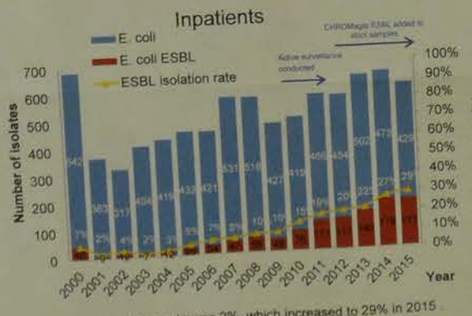


All strains were sensitive to carbapenem antibiotics, which are first-line agents for treating ESBL. Some showed resistance to FOM and ST, which are prescribed for UTI treatment. Moreover, CAZ- and AZT-sensitive strains were detected, highlighting the importance of awareness.

**E. coli* ESBL isolates increased compared to other ESBL-producing bacteria. Therefore, we conducted detailed analysis of the inpatient and outpatient isolation rates and class of isolated specimens of *E. coli* ESBL.

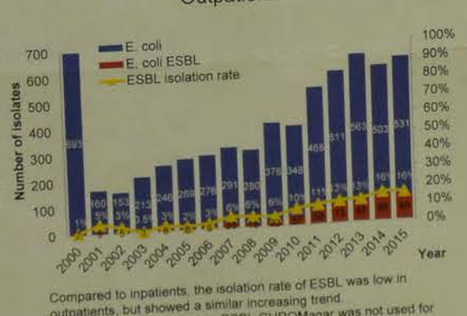
Isolation rates of *E. coli* ESBL

Inpatients



In 2001 the isolation rate was 2%, which increased to 29% in 2015.

Outpatients



Compared to inpatients, the isolation rate of ESBL was low in outpatients, but showed a similar increasing trend.
*Unlike in inpatient samples, ESBL CHROMagar was not used for stool samples.

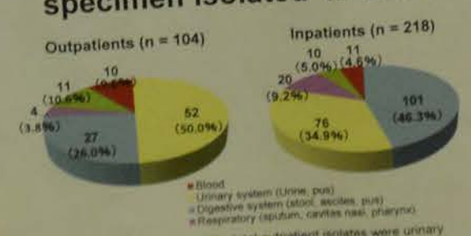
Genetic result of *E. coli* ESBL at our hospital



1997: Genetic results for ESBL genes detected from our hospital
2011: Genetic results for ESBL genes during consideration of culture media
2014: Genetic results for 57 strains selected randomly

Among the previously genetically analyzed strains, CTX-M2 was the only gene detected in 1997; however, in 2011 and 2014, CTX-M1 and CTX-M9 were prevalent.

Proportion of each *E. coli* ESBL specimen isolated in 2015.



The results showed that 50% of the total outpatient isolates were urinary system-derived, which were all isolated from urine specimens. For inpatients, most specimens were from the digestive system, and were mostly stool isolates. Furthermore, 4.6 and 9.6% of inpatient and outpatient isolates, respectively, were from blood specimens.

Source of infections and antibiotic-resistant *E. coli* ESBL isolates detected in blood cultures in 2015 (21 cases)

Case	Sex	Patient	Infection Website (Underlying disease)	Isolated, other specimen	Final choice antimicrobials Treated		Outcome	Risk ESBL resistant antimicrobial agents
1	M		UTI(hypertrophy)	urine	PIPC/TAZ		cure	CM, LVFX
2	M		cholangitis	-	CPFM	MEPM	cure	-
3	M		UTI	urine	MEPM	MEPM	cure	LVFX
4	M		pyelonephritis	urine	MEPM		cure	CM, LVFX
5	M		UTI	urine	PIPC/TAZ		cure	CM, LVFX
6	M		UTI(bladder cancer)	urine	MEPM		cure	CM, LVFX, ST
7	M		pyelonephritis	urine	LVFX		cure	CM, LVFX
8	M		UTI	urine	MEPM		cure	CM, LVFX
9	M		UTI	urine	MEPM	MEPM	cure	LVFX
10	M		UTI	urine	MEPM	MEPM	cure	LVFX, ST
11	M		UTI	urine	LVFX	LVFX	cure	CM, LVFX
12	M		UTI(malignant lymphoma)	urine	MEPM	MEPM	cure	-
13	M		UTI	-	CTRX		cure	LVFX, ST
14	M		cholecystitis	-	PIPC/TAZ		cure	LVFX, ST
15	M		UTI(renal cyst)	-	MEPM	MEPM	cure	CM, LVFX
16	M		UTI	urine	MEPM	MEPM	cure	CM, LVFX
17	M		UTI(urolithiasis)	urine	MEPM	MEPM	cure	CM, LVFX
18	M		UTI(urolithiasis)	abdominal cavity drain	IPM	IPM	cure	CM, LVFX, FOM
19	M		UTI(pancreatic cancer)	blood	MEPM	MEPM	cure	CM, LVFX
20	M		alcoholic hepatitis	urine, blood	LVFX, MINO	LVFX, MINO	cure	CM, LVFX
21	M		ureteral infection	fistula	VCM, CEZ	VCM, CEZ	cure	CM, LVFX

CM: Cefmetazole
LVFX: Cefazolin
MINO: Minocycline
PIPC/TAZ: Piperacillin-tazobactam
CEZ: Cefazolin
CTRX: Ceftriaxone
CPFM: Cefoperazone-sulbactam
FOM: Fosfomycin
UTI: urinary tract infection

UTIs were the most common infectious diseases. ESBLs were also isolated from urine specimens. Some strains were resistant to ST and FOM, which are generally prescribed to patients with UTI caused by ESBL-producing bacteria.

Comparison of isolation rates Our hospital and JANIS

		2011	2012	2013	2014
Third generation cephalosporins resistant *K. pneumoniae*	Teikyo	0.47%	0.49%	0.32%	0.38%
	JANIS	0.24%	0.24%	0.23%	0.33%
Third generation cephalosporins resistant *E. coli*	Teikyo	1.75%	2.02%	2.37%	3.20%
	JANIS	1.14%	1.30%	1.40%	1.82%
Fluoroquinolones resistant *E. coli*	Teikyo	3.01%	2.79%	3.00%	3.60%
	JANIS	2.52%	2.87%	3.12%	3.33%

JANIS: Japan Nosocomial Infections Surveillance

The isolation rate for third-generation cephalosporin-resistant *E. coli* increased at both Teikyo University Hospital and from JANIS. However, the rate was imperceptibly higher at our hospital.

Conclusion

- Isolation rates of ESBL-producing bacteria increased for *E. coli*, but not for *K. pneumoniae* and *P. mirabilis* strains.
- The resistance to antibiotics by *E. coli* ESBL in 2015 was as follows: : LVFX (76%), FOM (17.1%), ST (47.3%). Conducting drug susceptibility tests and confirming drug effectiveness are important for outpatient infection treatments.
- The high isolation rates of ESBL at our hospital are thought to have resulted from attentive implementation.
- The detection rates in outpatients were high, with various ESBL gene types, indicating that community acquisition should be considered.
- Continuous observation for drug-resistant bacteria is necessary for healthcare-associated infection control.

Microbiology

PA-66

Studies of isolation methods for diagnosis of *Chlamydophila pneumoniae* infections -approach for establishing a standardized method-

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Department of Medical Technology, School of Health Sciences, Kyorin University

KYORIN

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Abstract

【Aim】 A unified method is needed for routine work and epidemiological studies of *Chlamydophila pneumoniae* (*C. pneumoniae*) infections. The study aims to establish a standardized method for isolation of *C. pneumoniae* organisms.

【Materials & Methods】 Three experiments were conducted to determine the optimal conditions of *C. pneumoniae* isolation: 1) The effect of swabs of raw-materials (rayon for PL65 and BD BBL Culture Swab Plus™, polyester for BD-UVT) and transport medium (SPG and BD-UVT) on the number of inclusions; 2) To determine the cell group with the highest sensitivity to *C. pneumoniae*; number of inclusions and content of *C. pneumoniae* specific DNA were compared among five different cell cultures: HL, HeLa229, Hep-2, BICR18 and ChaGo K-1, respectively; and 3) HL cell cloning was done to get the highest sensitive cell to *C. pneumoniae* by a limiting dilution method.

【Results】 Similar sizes and number of inclusions were obtained in both SPG and commercially available transport medium: BD-UVT. A few inclusions were counted in the combination of PL65 swab (made of rayon) and BD-UVT. HL cells were categorized into 29 cell groups. The result was that the number of inclusions was BICR18 > HL > Hep-2 ≒ HeLa229 > ChaGo K-1 > HL-KG22. The sizes of inclusions were BICR18 > HL ≒ HL-KG1 and they were definitely larger than that in HL-KG22 and ChaGo K-1. Many large-sized inclusions were observed in HL-KG1 cells and BICR18 cells. There were no discrepancies between the number of inclusions and DNA content determined by real-time PCR.

【Conclusion】 Results show that swabs of raw-materials were not so effective on the number of inclusions formed when SPG was a transport medium. Although BICR18 cells formed many inclusions, they were not easy to make into monolayers. We recommend the use of SPG (or BD-UVT) for transport and stored mediums, and HL-KG1 cells for isolation.

Experiment 1

Materials & Methods

Cells: Three cell strains (HL-, Hep2- and HeLa229 cells) and two cell strains (BICR18- and ChaGo K-1 cells) were provided by NIID, Japan and purchased from European Collection of Authenticated Cell Cultures (ECACC) , respectively. The former cells were passaged every 3 or 4 days in 25 cm² culture bottles (Corning) in Eagle's minimum essential medium (MEM, Nissui Pharmaceuticals Company, Tokyo) containing 8% fetal bovine serum (FBS), 2 mM glutamine, 0.1% $NaHCO_3$ and 40 μg/mL gentamicin as cell culture medium (CCM). The CCM containing 0.04% glucose and 1μg/mL cycloheximide (CPGM) was used for evaluation of sensitivity of cells to *C. pneumoniae*. Cloned HL cells (KG1~KG22, etc.) were grown in a medium the same as the constitution of HL cells. The later cell strains (BICR18- and ChaGo K-1 cells) were cultured with the medium prepared by the company. BICR18 cells were larynx squamous cell carcinoma (lymph node metastasis). The culture medium was DMEM containing 10% FBS, 0.4 μg/mL hydrocortisone. ChaGo K-1 cells were from human lung bronchus carcinoma. The culture medium was RPMI1640, 1 mM sodium pyruvate (NaP), 2 mM glutamine and 10% FBS.

The effect of swabs of raw materials (rayon for PL6S and BD BBL Culture swab Plus™, polyester for BD-UVT) and transport medium (SPG, 2SP, BD-UVT and skim milk) on the number of inclusions were investigated. Each swab was spotted with 10 μL of *C. pneumoniae* suspension (1.8×10^6, 1.8×10^4, 1.8×10^3 IFU/mL) and the swab was soaked in transport medium. These were thoroughly vortexed and immediately inoculated on HL cell monolayers (Fig 1-1). The cells were cultured at 35.5°C. After 3 days culture, cells were fixed with methanol and stained with immune-peroxidase-AEC method (IP method)*. Number of inclusions were counted and compared among various conditions.

3 or 4 days culture of HL cells were treated with EDTA-trypsin to prepare cell suspension in CCM

↓

Two groups of cell monolayers in 24 wells plates were infected with *C. pneumoniae* suspension

↓

Centrifuged at 1,600 × g for 60 min.

↓

One group was aspirated inoculum and new medium (CPGM) was added for culture of *C. pneumoniae*

↓

Culture at 5% CO_2, 35.5°C for 3 days

↓

Each monolayer cell was fixed with methanol and stained with IP method. The other groups of cells were harvested together with the culture medium for determination of DNA content

Fig. 1-1 Procedure for infectivity assay of *C. pneumoniae*

* Target gene: *ompA* (Apfalter P et al, J Clin Microbiol 2003,Vol41)

forward primer QMOMP1

: 5'-GATCCGCTGCTGCAAACTATACT-3'

reverse primer QMOMP2

: 5'-GTGAACCACTCTGCATCGTGTAA-3'

TaqMan probe QMOMPS

: 5'-TAGGCCGGGTTAGGTCTATCTACGGCAGT-3'

* Standard curve was prepared ranging from 10 to 10^4 copies per reaction

Detailed methods could not be described here. If knowing detailed information is necessary, please contact the corresponding author (E-mail: komo@ks.kyorin-u.ac.jp)

Fig. 1-2 Assay procedure for real-time PCR

Result

Table 1. Effect of raw swabs and transport mediums on the growth of *C. pneumoniae* using HL cell cultures

C. pneumoniae IFU/10μL/well	swab product name	swab raw	transport medium kind	transport medium volume for use	blind passage 1st	blind passage 2nd	blind passage 3rd
18,500	Not used		SPG	1 mL	+	+	+
185					-	+	+
19					-	+	+
18,500	PL 6S	rayon	SPG	1 mL	-	-	+
185					-	-	+
19					+	+	+
18,500	BD BBL culture swab plus single	rayon	SPG	1 mL	-	+	+
185					-	+	+
19					+	+	+
18,500	Not used		BD-UVT	3 mL	-	+	+
185					-	+	+
19					+	+	+
18,500	BD-UVT swab	polyester	BD-UVT	3 mL	-	+	+
185					-	+	+
19					-	-	+
18,500	PL 6S	rayon	BD-UVT	3 mL	-	-	+
185					-	-	-
19					-	-	-

* Ten microliters of *C. pneumoniae* suspension was dropped on the swabs and then the swabs were soaked into transport medium (SPG, BD-UVT). Similar levels of DNA copies were estimated in SPG and BD-UVT when the swabs were soaked into 1 mL of BD-UVT. However, the infectivity level of PL6S swab samples was lower than that of BD-UVT. The PL6S swab seems to inhibit *C. pneumoniae* infection.

Table 2. Effect of swabs and / or transport medium on DNA contents determined by real-time PCR

sample	IFU/assay	transport medium	volume	copies of C.pn (MOMP Target)
BD	3.40×10^3	BD-UVT	3 mL	2.13×10^3
			1 mL	8.42×10^3
			0.5 mL	2.06×10^4
SPG		SPG	3 mL	3.57×10^3
			1 mL	7.00×10^3
			0.5 mL	5.90×10^3
BD	3.40×10^2	BD-UVT	3 mL	6.89×10^2
			1 mL	1.59×10^3
			0.5 mL	2.64×10^3
SPG		SPG	3 mL	1.79×10^3
			1 mL	1.85×10^3
			0.5 mL	3.61×10^3

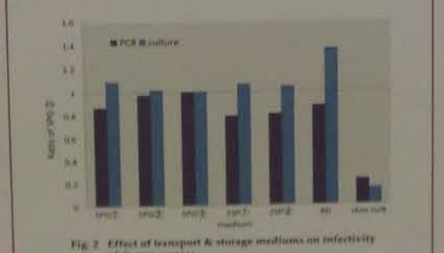


Fig. 2 Effect of transport & storage mediums on infectivity of *C. pneumoniae*

Experiment 2

HL cell cloning and STR-PCR (short tandem repeat-PCR) were preformed to get the highest sensitive cells to *C. pneumoniae* by the limiting dilution method, and to confirm the human cell strain authentication. Our HL cells were provided from NIID Japan and maintained at our laboratory for over 20 years. At first, we simply wanted to know the origin of HL cells, but there was an unexpected result. Therefore in order to eliminate the possibility of cell contamination, we got HL cells again just before this experiment. The HL cells accepted were passaged once and we prepared cell suspension (5×10^5 cell/mL) for according to the company's instructions.

Result

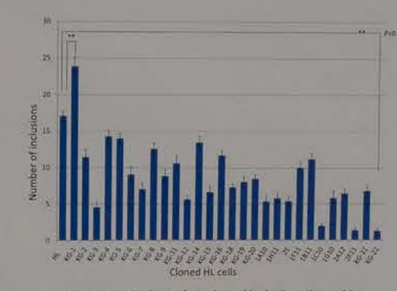


Fig. 3 Comparison of number of inclusions formed in cloned cells

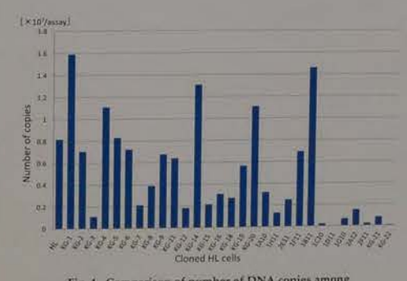


Fig. 4 Comparison of number of DNA copies among cloned cells determined by real-time PCR

Table 3. Result of authentication test for HL cell lines

Cell No.	Cell Name	EV*	D13S317	D7S820	D16S539	vWA	TH01	TPOX	CSF1PO	AMEL
	HL	1.000	11,12	8,12	9,10	16,18	7	12	9,10	X
ICRB 9004	HeLa	0.968	11,12	8,12	9,10	16,18	7	8,12	9,10	X
CCL-2	HeLa	0.968	11,12	8,12	9,10	16,18	7	8,12	9,10	X
RCB 0007	HeLa	0.930	11,12	12	8,12	9,10	16,18	7	8,12	9,10	X
JCRB 0538	TIG-109	0.667	11,12	8	12,13	9,11	16,18	7,9	8	9,10	X
KBN 0097-05	RERF-LC-AI	0.640	11	12	8,12	10	17	7	11	10	X
JCRB 0174.0	NCC16-P11	0.600	11,12	12	10,11	9,10	16,19	6,9	8	9,10	X
JCRB 1125	PL502	0.600	9,11	8,12	9,10	9,10	16,18	7	8	10,12	X
JCRB 1345	PALL-2	0.600	11,12	8	8,11	9,10	18,19	7,9	8	10	X,Y
JCRB 1416	KasumiA-S54	0.600	11,12	12	11	9,12	16,18	6,9	11,12	10,12	X
ICRB 1082	KYSE 170	0.581	11,12	9	11,12	9,10	16,17	7,9	11	10,11,13	X

* Evaluation value

Authentication of HL cell lines were performed using PowerPlex® systems (Promega) standardization of STR profiling

Experiment 3

To determine the cell group with the highest sensitivity to *C. pneumoniae*, the number of inclusions and content of *C. pneumoniae* specific DNA were measured. The cells used were HL, HeLa229, Hep-2, BICR18, ChaGo K-1 cell and cloned HL cell, respectively.

Result

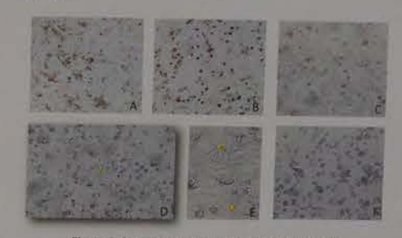


Fig. 5 Infection patterns : before and after staining

A (parent cell), B (KG-1), C (KG-22) : I-P staining (× 100)
D, E (KG-1), F (KG-22), D, F(× 100), E (× 200), *C. pneumoniae* KP-007 (clinical isolate)

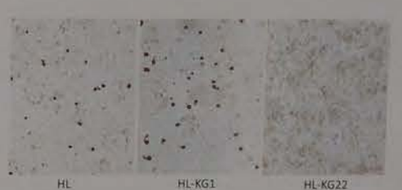


Fig. 6 Comparison of number of inclusions formed in HL, HL-KG1 and HL-KG22 cells

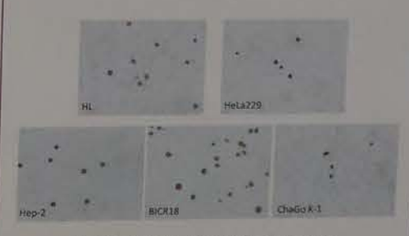


Fig. 7 Comparison of number of inclusions formed in five different cell strains

The number of inclusions was BICR18 > cloned HL (HL-KG1) > Hep-2 ≒ HeLa229 > ChaGo K-1 > HL-KG22

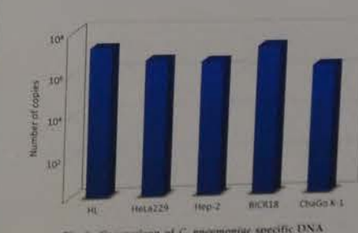


Fig. 8 Comparison of *C. pneumoniae* specific DNA content in five cell strains

Conclusion

1) Transport and stored medium, BD-UVT gave the same number of inclusions as well as SPG, resulting in the BD-UVT being able to use instead of SPG.
2) The raw swab materials (rayon for PL6S and BD-UVT) were not affected on the content of *C. pneumoniae* specific DNA copies determined by real-time PCR. However BD-UVT is better than PL6S in infectivity assays. It seems that the PL6S swab eluted something to inhibit *C. pneumoniae* infection.
3) The number of inclusions was BICR18 >HL-KG1 >HL > Hep-2 ≒ HeLa229 > ChaGo K-1.
4) The DNA contents for each cell were correlated with the results of inclusion counting.
5) BICR18 cells formed many inclusions with large sizes; however the cells were not easy to prepare into monolayers.
6) The use of BD-UVT and HL-KG1 cells for *C. pneumoniae* isolation may therefore be practical in routine work.

Acknowledgement

This work was supported by Tsukuba Medical Laboratory of Education and Research NPO Grant Number TMER/26.1.01.

Microbiology

PA-67

Analysis of the *thyA* gene of small-colony variants of *Escherichia coli*

Tetsuya Sugahara¹, Takuya Nakano¹, Tetsuya Nakano¹, Kenta Ishii¹, Yoshiaki Harada², Hiroyuki Nozaka¹, Chowdhury Rafiqul Hasan³, Hiroyuki Kaneko¹

1) Department of Laboratory Medicine, Hirosaki University Graduate School of Health Sciences, Japan
2) School of Health Sciences, Hirosaki University, Japan
3) Department of Microbiology, University of Dhaka, Bangladesh

Introduction

Small-colony variants (SCVs) are mutant strains that grow slowly and show atypical colony morphology. Thymidine-dependent SCVs (TD-SCVs) are dependent on thymidine for their growth (Fig. 1). In *Staphylococcus aureus*, the occurrence of TD-SCVs is mainly due to a mutation in *thyA*, the structural gene of thymidylate synthase. The aim of this study was to analyze the *thyA* sequences in TD-SCVs of *Escherichia coli*.

Methods

In this study, we used 5 strains of *E. coli* TD-SCVs (SH4149, SH4506, SH4995, SH6190, SH6244), which were obtained from clinical fecal samples. The revertants successfully grew on Mueller Hinton medium and showed a normal phenotype (Fig. 2). We sequenced the *thyA* of both TD-SCVs and their revertants.

Results

SH4506, SH6244 and SH6190 showed individual amino acid mutations, p.Leu44Gln, p.Trp133Arg, and p.Ser180Arg, caused by c.131T>A, c.397T>A, and c.540C>A, respectively. In SH4149, a nonsense mutation of p.Gln191X, produced by c.571C>T was observed. SH4995 contained a frameshift mutation caused by c.143_144ins with a 1201-bp fragment. Four revertants of the TD-SCVs, except for SH4995, were obtained. The revertant of SH6244 showed substitution of the same amino acid as the wild-type strain. The revertants of SH6190 and SH4149 showed substitutions to different amino acids. On the other hand, there was no difference in *thyA* sequence between SH4506 and its revertant, which was thymidine-independent (Table 1, Fig. 3).

Table 1. Mutations of *thyA* in TD-SCVs and compensatory *thyA* mutations in

	thyA Variant	ThyA
SH4149	c.571C>T	p.Gln191X
SH4506	c.131T>A	p.Leu44Gln
SH4995	c.143_144ins	fs
SH6190	c.540C>A	p.Ser180Arg
SH6244	c.397T>A	p.Trp133Arg



Fig. 3.
ThyA amino-acid sequences

Microbiology

PA-67

Analysis of *thyA* mutation in thymidine-dependent small-colony variants of *Escherichia coli*

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1) Department of Laboratory Medicine, Shinshu University Hospital
2) School of Health Sciences, Faculty of Medicine, Gunma University

Introduction

Small-colony variants (SCVs) are mutant strains that grow slowly and show atypical colony morphology. Thymidine-dependent SCVs (TD-SCVs) are dependent on thymidine for their growth (Fig. 1). In *Staphylococcus aureus*, the occurrence of TD-SCVs is mainly due to a mutation in *thyA*, the structural gene of thymidylate synthase. The aim of this study was to analyze the *thyA* sequences in TD-SCVs of *Escherichia coli*.

Methods

In this study, we used 5 strains of *E. coli* TD-SCVs (SH4149, SH4506, SH4995, SH6190, SH6244), which were obtained from clinical fecal samples. The revertants successfully grew on Mueller Hinton medium and showed a normal phenotype (Fig. 2). We sequenced the *thyA* of both TD-SCVs and their revertants.

Results

SH4506, SH6244 and SH6190 showed individual amino acid mutations, p.Leu44Gln, p.Trp133Arg, and p.Ser180Arg, caused by c.131T>A, c.397T>A, and c.540C>A, respectively. In SH4149, a nonsense mutation of p.Gln191X, produced by c.571C>T was observed. SH4995 contained a frameshift mutation caused by c.143_144ins with a 1201-bp fragment. Four revertants of the TD-SCVs, except for SH4995, were obtained. The revertant of SH6244 showed substitution of the same amino acid as the wild-type strain. The revertants of SH6190 and SH4149 showed substitutions to different amino acids. On the other hand, there was no difference in *thyA* sequence between SH4506 and its revertant, which was thymidine-independent (Table 1, Fig. 3).

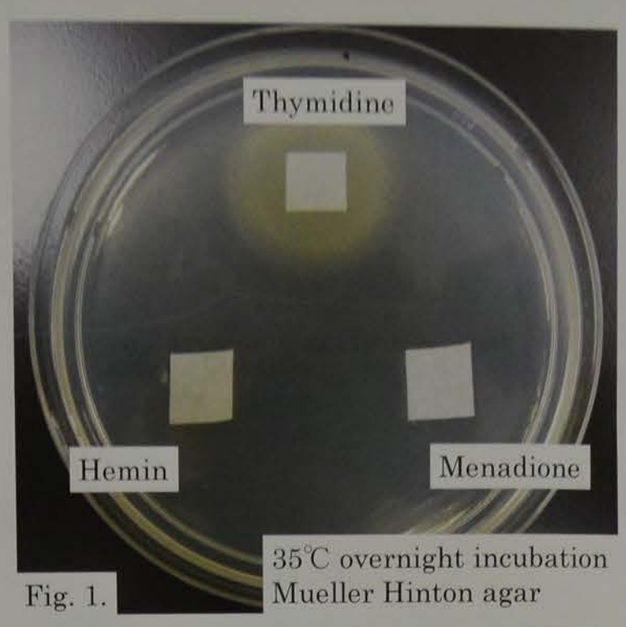


Fig. 1. 35℃ overnight incubation Mueller Hinton agar

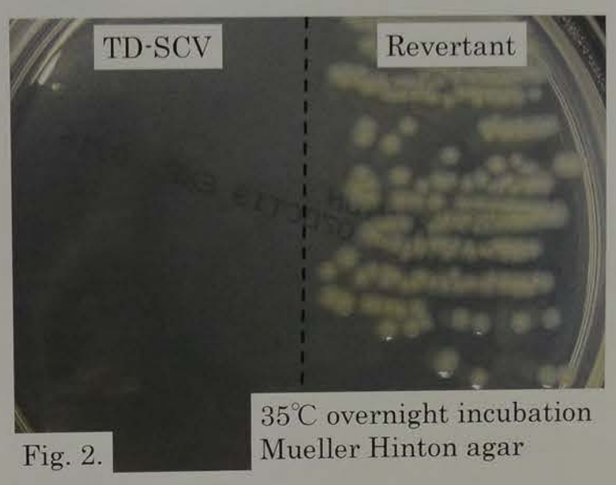


Fig. 2. 35℃ overnight incubation Mueller Hinton agar

Conclusions

In *E. coli*, *thyA* mutations are a major cause of the occurrence of TD-SCVs, similar to that in *S. aureus*. However, other factors in addition to *thyA* mutation may result in TD-SCVs because one isolate showed no difference in the *thyA* sequence between the TD-SCV and the revertant.

Table 1. Mutations of *thyA* in TD-SCVs and compensatory *thyA* mutations in revertans

	Variant		Revertant	
	thyA	ThyA	*thyA*	ThyA
SH4149	c.571C>T	p.Gln191X	c.571C>T c.572A>G	p.Gln191Trp
SH4506	c.131T>A	p.Leu44Gln	c.131T>A	p.Leu44Gln
SH4995	c.143_144ins	fs	-	-
SH6190	c.540C>A	p.Ser180Arg	c.538A>G c.540C>A	p.Ser180Gly
SH6244	c397T>A	p.Trp133Arg	Wild type	Wild type



Fig. 3. ThyA amino-acid sequences of *E. coli* TD-SCVs and revertants

Shinshu University Hospital

Microbiology

PA-68

Poster Number
PA-68

The effect of farnesol on virulence factors of clinical isolates of *Pseudomonas aeruginosa*

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Yoko Mano[1], Nobuhiko Furuya[1]
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Revised Abstract

Back ground: *Pseudomonas aeruginosa* is the most common Gram-negative pathogen causing nosocomial pneumonia, and it is frequently implicated in hospital-acquired urinary tract and bloodstream infections. It is frequently found in mixed infections with the yeast, *Candida albicans*. The fungus produces farnesol activities as an effector in interspecies interaction. In this study, we investigated how the *C. albicans* farnesol might affect virulence factors of *P. aeruginosa* clinical isolates, such as pyocyanin, elastase and protease.

Method: The effects of farnesol on virulence factors of *P. aeruginosa* clinical isolate were phenotypically tested. Pyocyanin was extracted from culture supernatants and its absorption at 520nm was measured. Elastase and total protease activities were determined using the Elastin Congo red (Sigma) assay and the Remazol Brilliant Blue-Hide (Sigma) assay, respectively.

Result: *C. albicans* culture supernatants reduced the production of *P. aeruginosa* pyocyanin by 65.5%. Pyocyanin production levels in all *P. aeruginosa* culture supernatants in the presence of farnesol were lower than those in the absence of farnesol. The reduction rates of elastase and total protease production in the *P. aeruginosa* culture supernatants in the presence of farnesol had a broad range (0-41%) and the mean elastase and total protease production levels within *P. aeruginosa* culture supernatants with farnesol were tended to be produce lower than those in absence of farnesol, respectively.

Conclusion: Exogenous addition of farnesol resulted in decreases of *P. aeruginosa* exoproducts. These results suggest that farnesol may contribute to the decrease of *P. aeruginosa* pathogenesis.

Materials and Methods

Bacterial Strains, media and culture conditions. *Pseudomonas aeruginosa* PAO1 and ten clinical isolated strains in this study; 5 strains were pus (Pa1 - Pa5) and 5 were urine(Pa6 – Pa10) *P. aeruginosa*, *Candida albicans* ATCC90028. *P. aeruginosa* overnight cultures were grown in 5mL Mueller-Hinton Broth (MHB) at 37℃ on the shaking incubator and, when needed, farnesol were provided at the following concentrations 1mM. *C. albicans* overnight cultures were grown in 5mL MHB at 37℃ on the shaking incubator.

Pyocyanin Production assay. Pyocyanin was extracted from culture supernants and measured by the method of Essar et al. A 3 mL chloroform was added to 5 mL of culture supernants and mixed. The chloroform layer was transferred to a fresh tube and mixed with 1 mL of 0.2 M HCl. After centrifugation, the top layer (0.2M HCl) was removed and its absorption at 520 nm was measured[1)].

Elastase Production assay. LasB elastase testing was carried out using Elastin Congo Red (ECR, Sigma Chemical Company) (10 mg/mL) . Supernatant (0.5 mL) was mixed with 10 mg of ECR and 0.5 mL of ECR buffer (100 mM TriseHCl, pH 7.5). The mixture was then incubated for 6 h at 37° C with shaking. Following centrifugation to remove insoluble ECR, OD 495 nm was measured[2)].

Protease Production assay. Proteolytic activity was carried out with using Remazol Brilliant Blue R–Hide (Sigma Chemical Company)(1 mg/mL) . Supernatant (2mL) was mixed with 3mg of Remazol Brilliant Blue R–Hide and 1mL of Remazol Brilliant Blue R–Hide buffer (10mM TriseHCl, pH 7.5). The mixture was then incubated for 1 h at 37° C with shaking. Following centrifugation to remove insoluble Remazol Brilliant Blue R–Hide , OD 595 nm was measured[3)].

***P. aeruginosa* Growth assay upon farnesol treatment.** *P. aeruginosa* were inoculated in MHB supplemented with concentrations of 1mM farnesol. The survival rate cultures, expressed as colony forming units (CFU), was compared to farnesol free cultures[4)].

Results

Figure 1. Adding *C. albicans* supernatants, the amount of pyocyanin produced by *P. aeruginosa* clinical isolates was reduced.

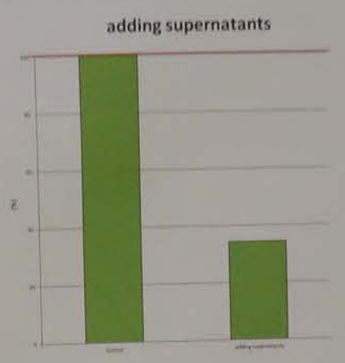


Figure 2. Production of pyocyanin were reduced in *P. aeruginosa* treated with farnesol

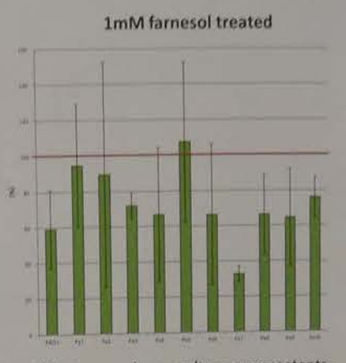


Figure 3. Elastase and protease production levels within *P. aeruginosa* culture supernatants with farnesol were tended to be produce lower than those in absence of farnesol

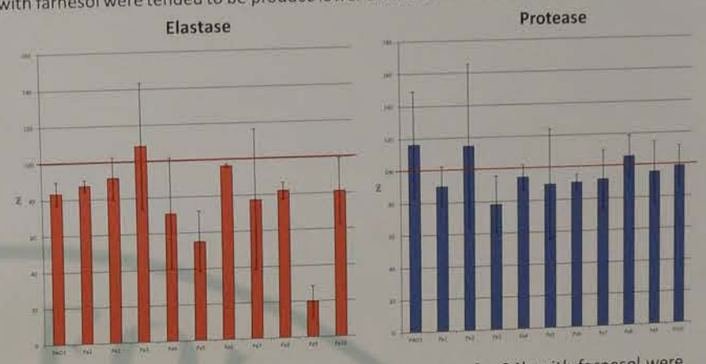
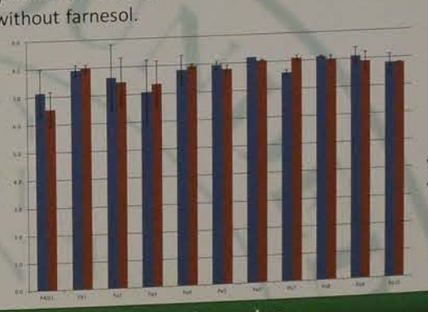


Figure 4. Colony count of *P. aeruginosa* isoalets grown for 24h with farnesol were similar to that without farnesol.



Discussion

- *C. albicans* farnesol inhibited the production of pyocyanin and a part of exotoxin of *P. aeruginosa*.
  - — Exotoxin was divided into two kinds of group ; either effected by farnesol or not.
- No significant effect of farnesol was observed against growth.
  - — It is suggested that the farnesol acted directly on the production of pyocyanin and a part of exotoxin.

Conclusions

- These date show that farnesol significantly inhibited the production of pyocyanin and elastase without signification inhibition of total protease.
- This study emphasizes the importance of no effect on growth inhibited in the presence of 1mM farnesol.

Selected References

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Conflicts of Interest : The authors declare no conflict of interest associated with this manuscript

PA-69

Identification of genes associated with penetration activity of the human type of *Edwardsiella tarda* EdwGII

○Chigusa Suezawa[1)], Masashi Yasuda[1)], Kiyoshi Negayama[2)], Taeko Kameyama[3)], Misato Hirauchi[3)], Toshihiro Nakai[4)], Jun Okuda[1)]

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[2)] Dept. of Clinical Laboratory, Kagawa Univ. Hosp.
[3)] Dept. of Central Clinical Laboratory, Kagawa Prefectural Central Hosp.
[4)] Graduate School of Biosphere Science, Hiroshima Univ.

Summary

Introduction: *Edwardsiella tarda* is a gram-negative pathogen with a broad host range including fish and humans. *E. tarda* causes edwardsiellosis in fish. In human, *E. tarda* causes gastrointestinal and extraintestinal infections. Several virulence factors have been identified to date including T3SS and T6SS. However, the exact infection mechanism, especially that in humans, cannot be explained based only on known virulence factors. We were interested in determining whether differences existed in the pathogenicities of human isolates and diseased fish isolates in humans. **Methods:** The penetration activity of 22 strains of *E. tarda*, including 10 human isolates and 12 diseased fish isolates, through Caco-2 cell monolayers were evaluated. To determine whether T3SS and T6SS were associated with penetration activity, we analyzed the distribution of the T3SS and T6SS-related genes by colony PCR. To identify genes responsible for penetration activity, we screened transposon (Tn) insertion mutants for reduced penetration activity. Motility assays, including swimming motility assay and swarming motility assay, were performed to determine the effects of Tn insertion on motility. We also carried out colony PCR to detect the *wecC* gene to differentiate the strains showing penetration activity. To investigate the virulence of *E. tarda* strains, we conducted the fly survival experiment. **Results:** All the human isolates showed penetration activity, while all fish isolates exhibited no penetration activity. There was no amplification for any of the tested T3SS and T6SS-related genes in the human isolates examined. Two Tn insertion mutants (mutant1 and mutant2) showed considerable reduction in penetration activity, and we identified the *wecC* and *fliF* genes as Tn insertion sites. The *wecC* mutant showed reduced motility as compared with that of the wild-type strain, while the *fliF* mutant exhibited immotility. The human isolates showed amplification of the *wecC* gene, whereas the fish isolates did not. There was no significant difference on survival rate of fly between *E. tarda* strains examined.

Conclusion

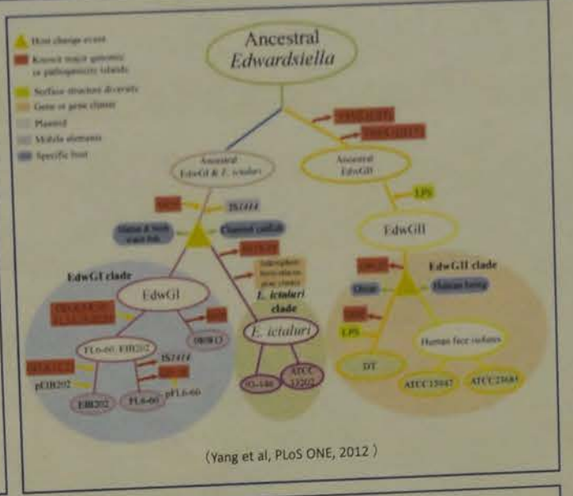
Motility mediated by the *wecC* and *fliF* genes appeared to be essential for penetration activity of *E. tarda* through Caco-2 cell monolayers. Furthermore, it was possible to group *E. tarda* strains into two types of human isolates and diseased fish isolates based on distribution of the *wecC* gene, T3SS and T6SS-related genes. As there was no significant difference on survival rate of fly between *E. tarda* strains examined, to clarify relationship between penetration activity and virulence of *E. tarda*, survival experiment using other insects instead of the fly or animals will be required.

Table 1 *Edwardsiella tarda* strains used in this study

Strains	Source	Other clinical information
EtCB1	human clinical specimens: blood	76-yr-old female with recurrent cholangitis
EtCB2	human clinical specimens: blood	83-yr-old female with suppurative cholangitis
EtK1	human clinical specimens: wound	83-yr-old male with burn
EtK2	human clinical specimens: drain fluid	77-yr-old male with rectal cancer
EtK3	human clinical specimens: drain fluid	70-yr-old male with rectal cancer
EtK4	human clinical specimens: pus	13-yr-old male with perirectal abscess
EtK5	human clinical specimens: ascites fluid	84-yr-old male with cecal cancer
EtK6	human clinical specimens: pus	80-yr-old male with rectal cancer
EtK7	human clinical specimens: drain fluid	65-yr-old male with acute pancreatitis
NBRC105688	human feces	
FK1051	Japanese flounder	
NUF82	Japanese flounder	
PE113	Japanese flounder	
E01-33	Japanese flounder	
E01-37	Japanese flounder	
NE8003	Japanese flounder	
E01-17	Red sea bream	
E01-40	Red sea bream	
REF179	Red sea bream	
REF146	Red sea bream	
REF0001	Red sea bream	
MEE0309	Red sea bream	

Table 2 Detection of genes associated with T3SS and T6SS

Strains	T3SS-related genes					T6SS-related genes	
	esaJ	eseE	eseB	esrA	esrB	evpB	evpC
EtCB1	-	-	-	-	-	-	-
EtCB2	-	-	-	-	-	-	-
EtK1	-	+	-	-	-	-	-
EtK2	-	-	-	-	-	-	-
EtK3	-	-	-	-	-	-	-
EtK4	-	-	-	-	-	-	-
EtK5	-	-	-	-	-	-	-
EtK6	-	-	-	-	-	-	-
EtK7	-	-	-	-	-	-	-
NBRC 105688	-	-	-	-	-	+	+
FK1051	+	+	+	+	+	+	+
NUF82	+	+	+	+	+	+	+
PE113	+	+	+	+	+	+	+
E01-33	+	+	+	+	+	+	+
E01-37	+	+	+	+	+	+	+
NE8003	+	+	+	+	+	+	+
E01-17	-	+	+	+	+	+	+
E01-40	-	+	+	+	+	+	+
REF179	-	+	+	+	+	+	+
REF146	-	+	+	+	+	+	+
REF0001	-	+	+	+	+	+	+
MEE0309	-	+	+	+	+	+	+



(Yang et al, PLoS ONE, 2012)

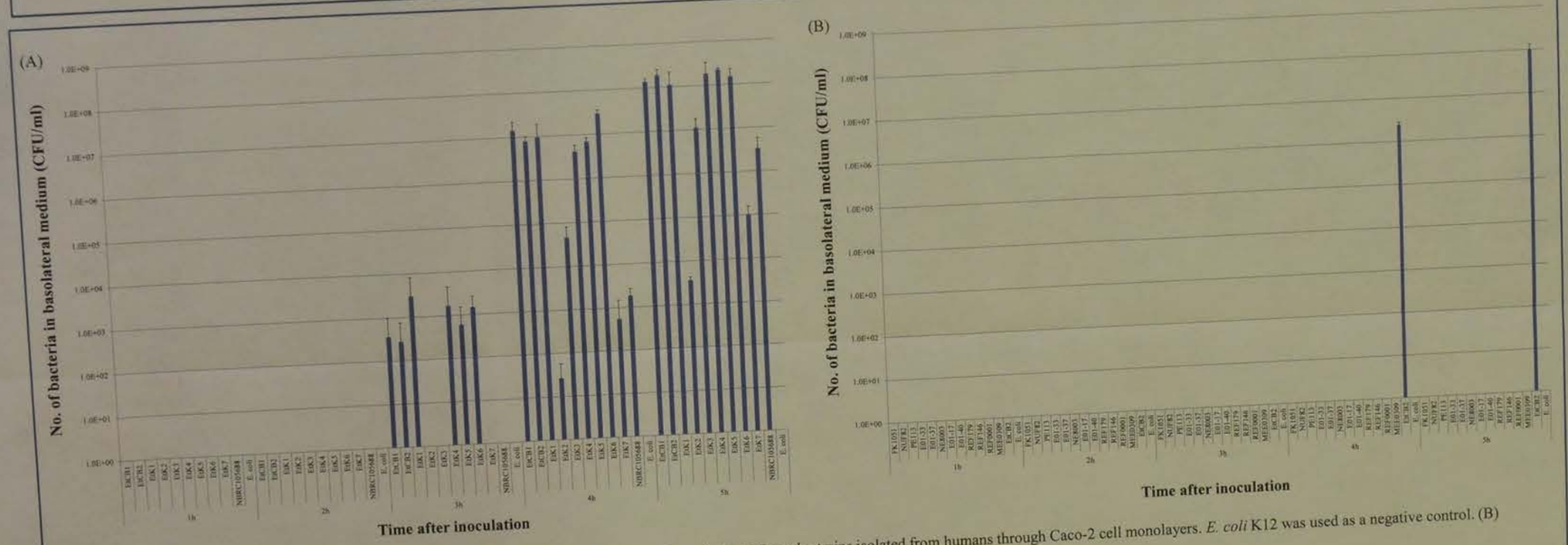


Fig. 1 Penetration activity of *E. tarda* strains isolated from humans and fish. (A) Penetration activity of *E. tarda* strains isolated from humans through Caco-2 cell monolayers. *E. coli* K12 was used as a negative control. (B) Penetration activity of *E. tarda* strains isolated from fish through Caco-2 cell monolayers. *E. tarda* EtCB2 was used as a positive control.

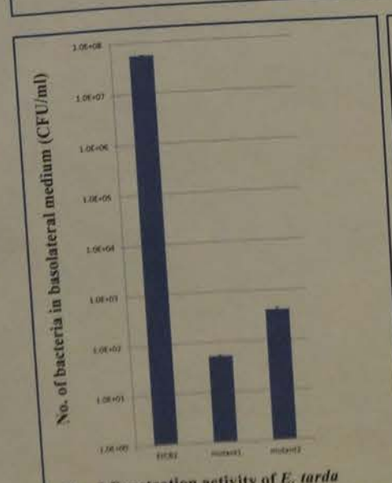
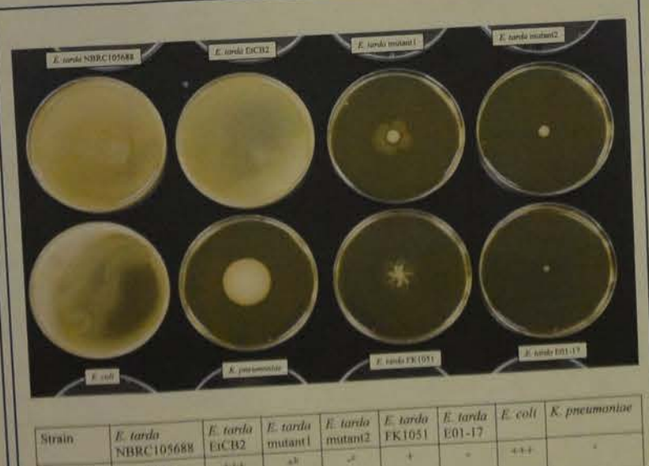


Fig. 2 Penetration activity of *E. tarda* mutant strains. Bacteria were inoculated onto the apical surfaces of Caco-2 cell monolayers at a MOI of 10, and the bacterial number in the basolateral medium was counted at 5 hours after inoculation. The assay was performed in triplicate, and the results are expressed as the mean ± SD.



Strain	*E. tarda* NBRC105688	*E. tarda* EtCB2	*E. tarda* mutant1	*E. tarda* mutant2	*E. tarda* FK1051	*E. tarda* E01-17	*E. coli*	*K. pneumoniae*
Swimming motility	+++[a]	+++	+[b]	-[c]	+	-	+++	-

Fig. 3 Swimming motility assay of *E. tarda*. *E. coli* and *K. pneumoniae* were included as positive and negative controls, respectively. [a]: Strongly positive, [b]: Slightly positive, [c]: Negative.



Strain	*E. tarda* NBRC105688	*E. tarda* EtCB2	*E. tarda* mutant1	*E. tarda* mutant2	*E. tarda* FK1051	*E. tarda* E01-17	*E. coli*	*K. pneumoniae*
Swarming motility	+++[a]	+++	+[b]	-[c]	++	-	+++	-

Fig. 4 Swarming motility assay of *E. tarda*. *E. coli* and *K. pneumoniae* were included as positive and negative controls, respectively. [a]: Strongly positive, [b]: Positive, [c]: Negative.

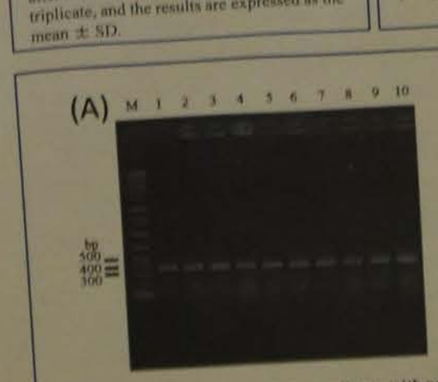


Fig. 5 Colony PCR to detect of the *wecC* gene with primers designed from the *wecC* gene sequence of *E. tarda* NBRC105688 isolated from humans. (A) Results of human isolates. Lanes: M, Molecular weight marker; 1, EtCB1; 2, EtCB2; 3, EtK1; 4, EtK2; 5, EtK3; 6, EtK4; 7, EtK5; 8, EtK6; 9, EtK7; 10, NBRC105688. (B) Results of diseased fish isolates. Lanes: M, Molecular weight marker; 1, FK1051; 2, NUF82; 3, PE113; 4, E01-33; 5, E01-37; 6, NE8003; 7, E01-17; 8, E01-40; 9, REF179; 10, REF146; 11, REF0001; 12, MEF0309; 13, NBRC105688. The size of the *wecC* gene amplicon was 384 bp.

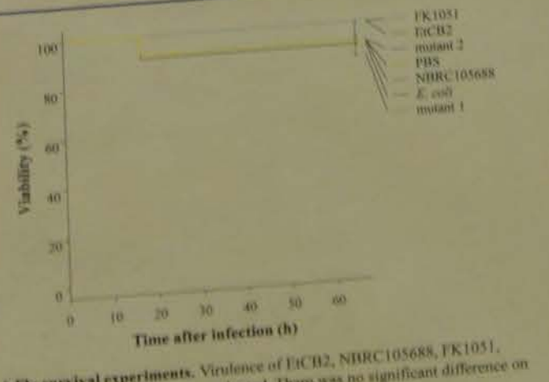


Fig. 6 Fly survival experiments. Virulence of EtCB2, NBRC105688, FK1051, mutant1, mutant2, and *E. coli* was evaluated. There was no significant difference on viability of fly between *E. tarda* strains examined.

Microbiology

PA-70

The association between virulence factors and viscosity of hypermucoviscosity phenotypes in *Klebsiella pneumoniae*

-Semiquantitative evaluation of viscosity using digital venire caliper-

Norihito Morimoto[1)], Yoshie Nishida[1,2)], Miyuki Morimoto[1)], Saya Kamioka[1)], Tamae Morita[1)], Katsumi Ogura[1)], Tetsuro Sugiura[1,2)] and Yoshihisa Matsumura[1,2)]

[1)]Department of Clinical Laboratory, Kochi Medical School Hospital and

[2)]Clinical Laboratory Medicine, Kochi University, Kohasu, Oko-cho, Nankoku, Kochi, 783-8505, Japan.

Background:

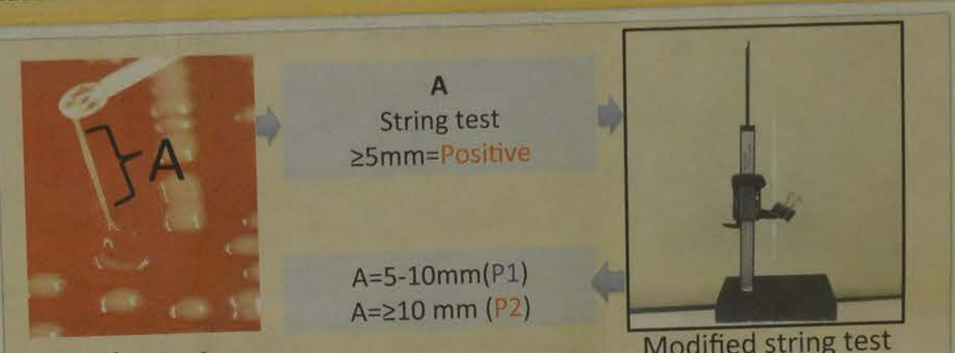
Klebsiella pneumoniae is normally found in the human intestine, and is the cause of respiratory tract and urinary tract infections. Hypermucoviscosity (HMV) phenotypes, particularly in serotypes K1 and K2, of *K. pneumoniae* have been associated with invasiveness and pathogenicity. This study aimed to investigate the association between the presence of virulence factors and viscosity in *K. pneumoniae*.

Materials & methods:

▪Samples

We examined 47 *K. pneumoniae* clinical isolates.

Samples	n
Sputum	17
Bronchoalveolar lavage fluid	2
Blood	12
Pus	6
Urine	4
Vagina swab	2
Pleural effusion	1
Synovial fluid	1
Feces	2
Total	47



▪PCR

Screening for capsular serotype (K1, K2 and K5) and virulence factor genes (*magA*, *rmpA*, *uge* and *kfu*) was determined by PCR.

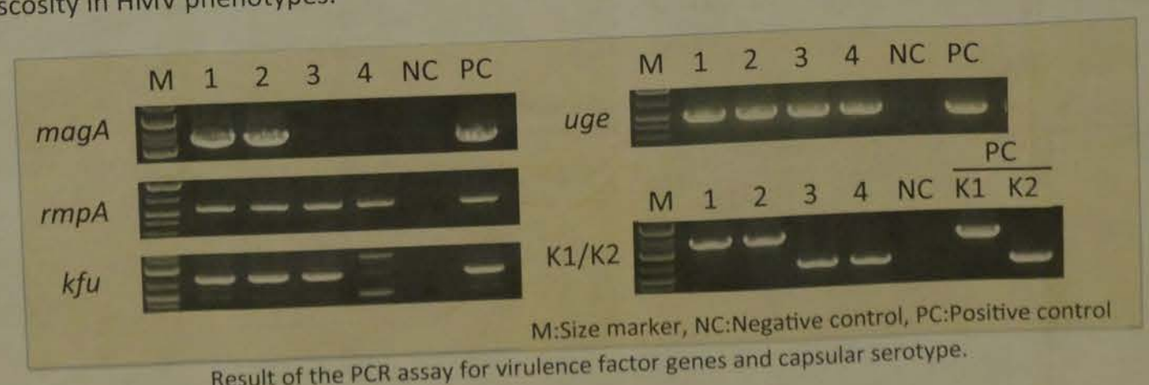
▪Determination of HMV phenotypes

HMV phenotypes were determined using the modified string test (a string measuring 5 mm or longer was defined as positive). Then, the viscous degree was classified as ≥10 mm (P2) or 5 - 10 mm (P1) using the digital venire caliper.

Results:

Sixteen of 47 isolates showed HMV phenotypes (P1: 5, P2: 11), and 10 of these were serotype K1 or K2. Serotype K1 or K2 was detected more frequently in P2 than P1 isolates. Regarding HMV phenotype, 15 (93.8%) and three (18.8%) isolates harbored *rmpA* and *magA*, respectively. Only one *rmpA*-positive isolate exhibited a non-HMV phenotype.

Nearly all HMV phenotypes of *K. pneumoniae* harbored *rmpA* and *uge*. However, more serotype K1 or K2 isolates exhibited high-degree viscosity in HMV phenotypes.



Result of the PCR assay for virulence factor genes and capsular serotype.

Factors associated with expression of HMV phenotype in *K. pneumoniae*

	No.of isolates (n=47)	HMV(Modified string test) Positive P1 (n=5)	Positive P2 (n=11)	Negative (n=44)	Univariate analysis (*P*) Positive vs Negative	P1 vs P2
virulence factor genes						
magA	3	1	2	0	< 0.01	0.931
rmpA	16	4	11	1	<0.0001	0.126
uge	41	4	11	26	<0.05	0.126
kfu	12	2	3	7	0.189	0.611
Capsular serotype						
K1	3	1	2	0	<0.01	0.931
K2	8	0	7	1	<0.001	<0.05

<0.05

Conclusion:

High-degree viscosity in HMV phenotypes of *K. pneumoniae* is more associated with capsular serotype K1 or K2 than virulence factor genes.

Microbiology

PA-71

PA-71 **Epithelial cell injury might differ according to the bacterial species; Cell viability in *P. aeruginosa* was reduced stronger than that in *E. coli* and *K. pneumoniae*.**

Misato Gorai[1,2], Natsumi Takeuchi[4], Kazue Fujita[3], Yoko Mano[4], Takahiro Suzuki[2], Yoshibumi Akatsu[2], Yoshinobu Saito[3], Akihiko Gemma[3], Nobuhiko Furuya[4]

[1] Bunkyo Gakuin University Graduate School, [2] Hitachi,Lid.Hitachi General Hospital
[3] Nippon Medical School, [4] Bunkyo Gakuin University, Tokyo, JAPAN

Revised Abstract

Background. Pneumonia is the leading cause of premature death in the world. The gram-negative bacilli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, are important causes of pneumonia in hospitalized and immunocompromised patients[1]. The lower respiratory tract is also a frequent locus of emerging infections, such as severe acute respiratory syndrome. Impaired epithelial barrier function correlate with disease severity of acute respiratory syndrome. However, little is known about the association between the extent of epithelial cell injury and the bacterial species. We hypothesized the extent of epithelial cell injury may differ according to the bacterial species. Here, we show in vitro study about the difference between the extent of epithelial cell injury and various bacterial species.

Materials and Methods. *P. aeruginosa*, *K. pneumoniae*, *Escherichia coli*, which represent common gram-negative pathogens that may contribute to nosocomial infection were evaluated in this study. A549 human airway epithelial cell viability assay, measurement of bacterial endotoxin and proteolytic activity of bacteria were performed to determine epithelial cell injury.

Results. Cell viability in *P. aeruginosa* was reduced stronger than that in *E. coli* and *K. pneumoniae*. Cell viability in stationary-phase *P. aeruginosa* was reduced stronger than that in log-phase *P. aeruginosa*. Proteolytic activity of *P. aeruginosa* increased more than that of *E. coli* and *K. pneumoniae*. However, levels of endotoxin did not significantly differ among these pathogens.

Conclusions. This study suggests that epithelial cell injury might differ according to the bacterial species. Impaired epithelial cells caused by *P. aeruginosa* are stronger than that by *E. coli* and *K. pneumoniae*. Furthermore, this difference is due to proteolytic activity of bacteria. Such information can support physicians' quick and accurate decision for diagnosis and treatment. There is concern that the subtle communication between the medical technologist microbiology and the infectious diseases physician would impact good patient care outcomes.

Materials and Methods

Bacterial Strains. *Pseudomonas aeruginosa* ATCC27853, *P. aeruginosa* PAO1, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* clinical isolated from sputum were used in this study.

Bacterial Growth. The inoculated bacterial culture of bacterial strains in LB broth, and incubated at 35℃ on a rotary shaker (130 rpm) for 24 hours. Then, the number of viable bacteria were quantified.

Cell Viability Assay. Bacterial strains were inoculated to 1.0 mL LB broth and grown with shaking at 35℃ 6 hours or 14 hours and bacteria-free culture supernatants were harvested. A549 human airway epithelial cells were grown in RPMI (Life Technologies Japan, Tokyo, Japan) containing 10% fetal bovine serum. The cells were placed in the wells of 96-well plates at a density of 1×10^4 cells/well, and the plates were incubated overnight under normal culture conditions (37℃ and 5% CO_2). After 1 hour of adjustment with serum-free medium, bacterial culture supernatants were added to the A549 cells. After 48 hours treatment, A549 cell viability was assessed using a Cell Counting kit-8 (Dojindo, Tokyo, Japan) according to the manufacturer's instructions [2].

Endotoxin Assay. Bacterial strains were inoculated to 1.0 mL LB and grown with shaking at 35 ℃ for 14 hours and bacteria-free culture supernatants were harvested. Endotoxin was assessed using a Limulus Color KY Test Wako (wako laboratory chemicals, Japan) according to the manufacturer's instructions.

Protease Assay. 10 μL of an overnight LB culture were spotted onto tryptic soy agar prate containing 2.0% skim milk. The plate were incubated at 37℃ for 24 hours. Proteolytic activity was detected as zone of clearing around the colonies [3].

Result

Figure 1. Bacterial growth did not significantly differ among these pathogens.

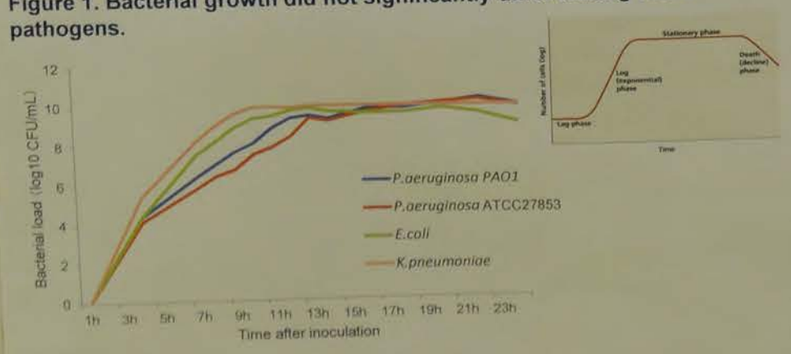


Figure 2. Cell viability in *P. aeruginosa* PAO1 was reduced stronger than that in *E. coli* and *K. pneumoniae*.

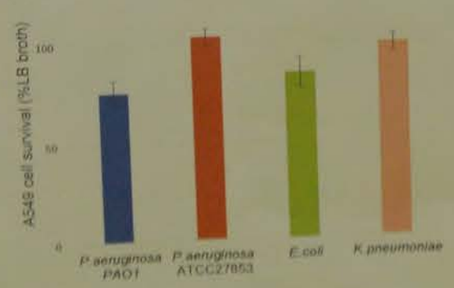


Figure 3. Cell viability in stationary-phase *P. aeruginosa* PAO1 was reduced stronger than that in log-phase *P. aeruginosa* PAO1.

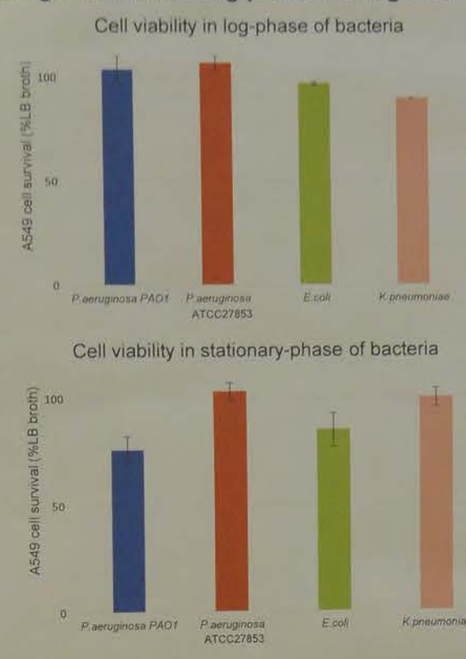


Table1. Proteolytic activity of *P. aeruginosa* increased more than that of *K. pneumoniae* and *E. coli* .

Bacterial strains	Zone of clearing around the colonies (mm)
P.aeruginosa ATCC 27853	23.4
P.aeruginosa PAO1	33.4
K.pneumoniae	-
E.coli	-

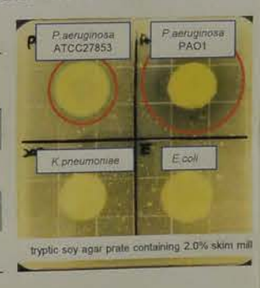
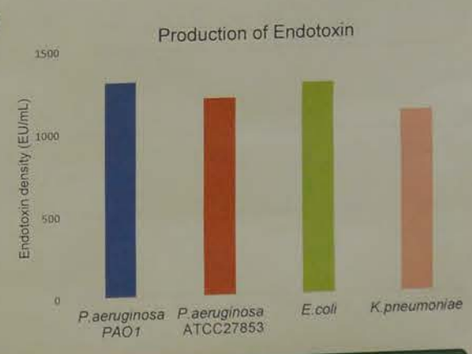


Figure 4. Endotoxin did not significantly differ among these pathogens.



Discussion

- Cell viability in *P. aeruginosa* was reduced stronger than that in *E. coli* and *K. pneumoniae*.
  - Protease activity may play a role in pathogenesis and cell viability
  - Protease activity vary between bacteria
  - Protease activity has been recognized as a virulence factor in the Gram-negative bacilli
  - Protease activity in Gram-negative bacilli has been linked with destruction of various mammalian cell lines
- However, which type of protease affects cell viability has been unsolved in this study.

Conclusions

This study suggests that epithelial cell injury might differ according to the bacterial species. Impaired epithelial cells caused by *P. aeruginosa* are stronger than that by *E. coli* and *K. pneumoniae*. Furthermore, this difference is due to proteolytic activity of bacteria. Such information can support physicians' quick and accurate decision for diagnosis and treatment. There is concern that the subtle communication between the medical technologist microbiology and the infectious diseases physician would impact good patient care outcomes.

Selected References

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2. Wataru Aoki: Effects of Clarithromycin against Pseudomonas aeruginosa from Chronic Respiratory Infection Patients with Long-term Clarithromycin Treatment. ICAAC 2014
3. Wood SE, Sample AK, Runyen-Janecky LJ: The sfa gene product required for Pseudomonas aeruginosa exotoxin A and protease production belongs to the cyclic AMP receptor protein family. J Bacteriol 1994, 176: 7532-42

Conflicts of Interest : The authors declare no conflict of interest associated with this manuscript

Microbiology

PA-72

PA-72 Identification and drug
strains isolated from o
OHitoshi Miyamoto, Shin
Yuri Tanaka, Takuya Ko
Department of Clinical Laborato

Purpose

Leptotrichia spp. which are one of oral microflora, have similar morphology to Fusobacterium nucleatum, but can grow under microaerophilic condition. Since it can cause sepsis in patients with blood disorders, it is very important to understand identification and drug susceptibility of clinical isolates.

This study was conducted to examine identification and drug susceptibility of Leptotrichia spp. strains isolated from oral specimens in Ehime University Hospital.

Methods

Between August 2015 to February 2016, seventeen strains were isolated from clinical oral specimens (11 bronchoalveolar lavage fluids and 6 oral abscess).

Isolates were identified using MALDI Biotyper (Bruker Daltonics)and 16S rRNA DNA sequencing. The Rapid ID 32A (bioMérieux) test was performed according to the manufacturer's instructions.

Antimicrobial susceptibility of benzylpenicillin (PCG),ampicillin(ABPC),sulbactam/ampicillin(S/A), tazobactam/piperacillin(T/P),ceftriaxone (CTRX), cefmetazole(CMZ), meropenem (MEPM), clindamycin (CLDM), and levofloxacin (LVFX) was checked using dry plates with Brucella broth after 46-48 hour incubation under anaerobic condition. All results were interpreted for "susceptible" and "resistance" including "intermediate".

Results

Using MALDI Biotyper, one strains was identified as Leptotrichia wadei, but other 16 strains were not accurately determined.

BLAST analysis of 16S rRNA DNA sequence demonstrated that one L. wadei strain identified by MALDI Biotyper had 99.46 % sequence similarity to L. wadei. Since sequence of other 16 strains showed less similarity than 98.7% (97.95-98.23%) to L. wadei, isolates could be new species. (Table 1)

Table 1. Identification of Leptotrichia spp. strains by MALDI Biotyper and 16S rRNA DNA sequencing

MALDI Biotyper	16S rRNA DNA sequencing	No. of isolates tested
Leptotrichia sp.	Similarity(Leptotrichia wadei LB16)	
Leptotrichia wadei	97.95~98.23	16
	99.46	1

Strains were identified as Leptotrichia sp. or Leptotrichia wadei using the MALDI Biotyper with high confidence scores of ≧2.0.

Microbiology

PA-72

PA-72

Identification and drug susceptibility of *Leptotrichia* spp. strains isolated from oral specimens

○Hitoshi Miyamoto, Shinobu Murakami, Mina Fukuoka
Yuri Tanaka, Takuya Kondo, Tatsuya Nishimiya

Department of Clinical Laboratory,Ehime University Hospital, Toon, Ehime, Japan

Purpose

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Results

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BLAST analysis of 16S rRNA DNA sequence demonstrated that one *L. wadei* strain identified by MALDI Biotyper had 99.46 % sequence similarity to *L. wadei*. Since sequence of other 16 strains showed less similarity than 98.7% (97.95-98.23%) to *L. wadei*, isolates could be new species. (Table 1)

Table 1. Identification of *Leptotrichia* spp. strains by MALDI Biotyper and 16S rRNA DNA sequencing

MALDI Biotyper	16S rRNA DNA sequencing Similarity (*Leptotrichia wadei* LB16)	No. of isolates tested
Leptotrichia sp.	97.95～98.23	16
Leptotrichia wadei	99.46	1

MALDI Biotyper : All strains were identified as *Leptotrichia* sp. or *Leptotrichia wadei* using the MALDI Biotyper, with high confidence scores of ≧2.0.

Isolated *Leptotrichia* sp. was different from *L. wadei* in *N* -acetyl-β-glucosaminidase and alkaline posphatase . (Table 2)

Table 2. Phenotypic characteristics of *Leptotrichia* spp. strains

Species	α-Gal	β-Gal	α-Glu	β-Glu	β-NAG	PAL	PyrA
L. buccalis	+	−	+	+	−	+	−
L. goodfellowii	−	+	−	+	+	+	
L. hofstadii	−	−	+	+	−	+	
L. hongkongensis	−	−	+	+	−	+	
L. shahii	−	−	+	−	−	−	
L. trevisanii	−	−	+	+	+	+	−
L. wadei	−	−	+	+	−	−	
Isolated strains (*Leptotrichia* sp.)	−	−	+	+	+	+	+

α/β-Gal : α/β-galactosidase , α/β-Glu : α/β-glucosidase , β-NAG : *N* -acetyl-β-glucosaminidase , PAL : alkaline posphatase , PyrA : pyroglutamic acid arylamidase

All strains showed susceptibility to of PCG, ABPC, S/A, T/P, CTRX, CMZ, MEPM, and CLDM, and resistance to LVFX. (Table 3)

Table 3. Antibiotic susceptibilities of *Leptotrichia* spp. strains

Agent	MIC (μg/mL) 0.12	0.25	0.5	1	2	4	8	16	32	64	128
PCG	17						-	-			
ABPC	11	6					-	-			
SBT/ABPC		-	-	17					-	-	
TAZ/PIPC				-	-	17					-
CTRX			-	-	17						-
CMZ			-	-	17				-	-	
MEPM	-	-	17							-	
CLDM		-	-	17							
LVFX		-	-					5	12	-	

"susceptible" | "intermediate" | "resistance"

Conclusion

- MALDI Biotyper and 16S rRNA DNA sequencing could not identify 16 of 17 isolates from oral specimens.
- Since there is possibility for new species of *Leptotrichia*, further investigations are needed.
- We should be aware of LVFX resistant *Leptotrichia* spp. strains.

COI

The authors declare no conflicts of interest.

Microbiology

PA-73

PA-73

FUKUSHIMA MEDICAL UNIVERSITY

Putative ligands for Dectin-2, a C-type lectin receptor in *Cryptococcus neoformans*

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Department of Clinical Laboratory, Fukushima Medical University[1]
Department of Medical Microbiology, Mycology and Immunology, Tohoku University Graduate School of Medicine[2]
Division of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University[3]

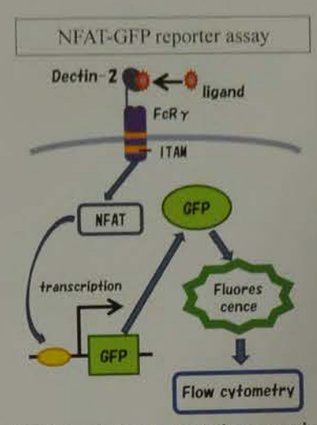
INTRODUCTION

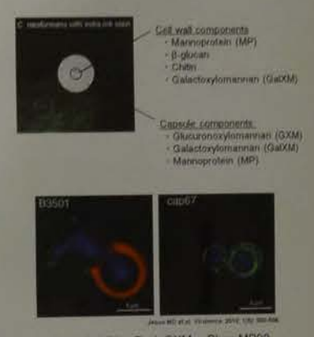
Cryptococcus neoformans, a yeast-form fungal pathogen, is rich in polysaccharides in its cell wall and capsule. Dectin-2 is a C-type lectin receptor (CLR) that recognizes high mannose polysaccharides. It is said that Dectin-2 is specifically expressed on macrophages and dendritic cells. Dectin-2 functions as a pattern recognition receptor (PRR) and plays a central role in immune response to fungal pathogens. Recently, we have analyzed the role of Dectin-2 in the host defense against *C. neoformans* infection. However characteristics of ligands recognized by Dectin-2 in this fungus have yet to be defined. Our aim is to explore the Dectin-2 ligands in *C. neoformans*.



METHODS

2B4-NFAT-GFP reporter cells expressing Dectin-2 were stimulated with B3501 (*C. neoformans* wild-type strain), Cap67 (acapsular mutant strain of B3501) or the disrupted lysates of these fungi for 24 h. *C. neoformans*-derived mannoprotein with a molecular weight of 98 kDa (MP98) was also used for this stimulation. The expression of GFP was analyzed in the reporter cells by flow cytometry. The influences of periodic acid (H_5IO_4) were analyzed. H_5IO_4 is known to break ring structure of the monosaccharides, including glucose, mannose and galactose, to linear structure, which leads to alteration of the polysaccharide structure.MP98 was kindly provided by Dr. S.M. Levitz (University of Massachusetts Medical School, Worcester, MA, USA).

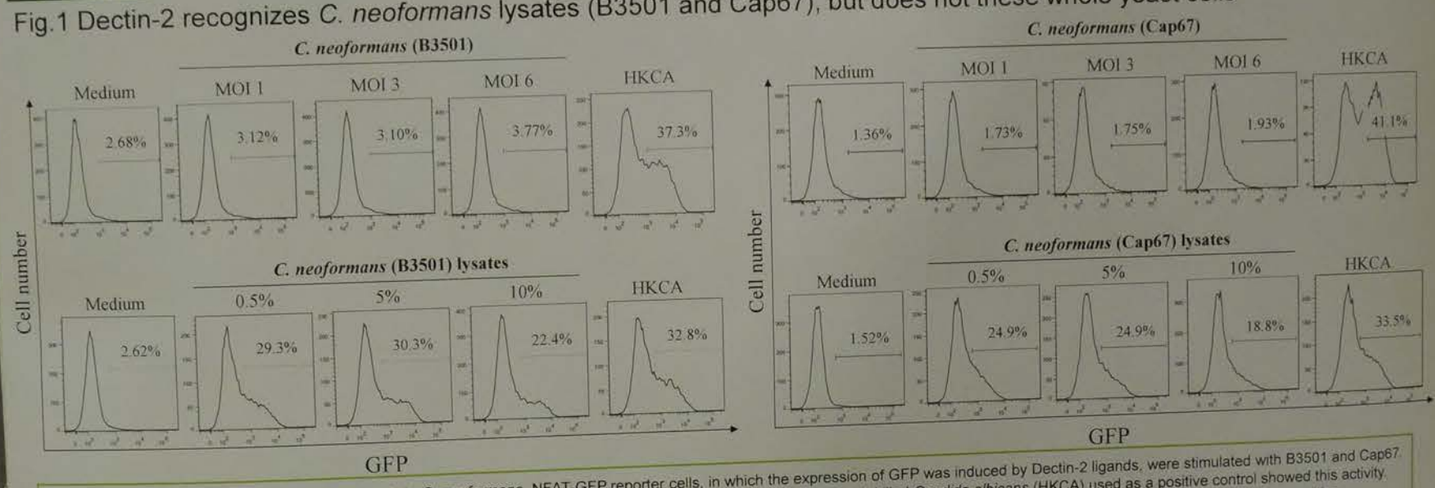




Green: GalXM, Red: GXM, Blue: MP98

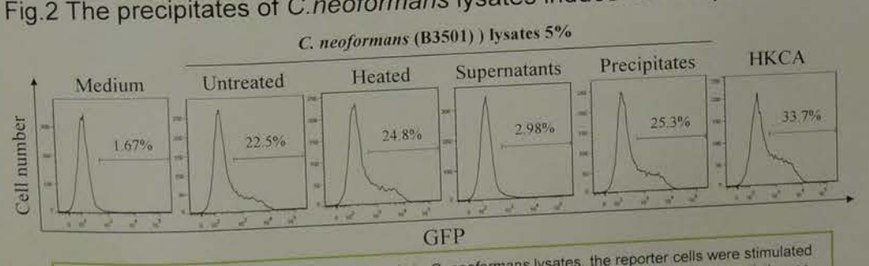
RESULTS

Fig.1 Dectin-2 recognizes *C. neoformans* lysates (B3501 and Cap67), but does not these whole yeast cells



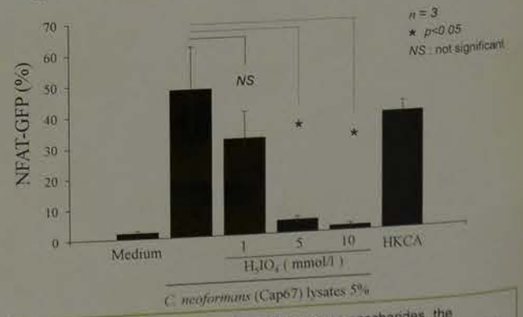
In order to confirm the presence of Dectin-2 ligands in *C. neoformans*, NFAT-GFP reporter cells, in which the expression of GFP was induced by Dectin-2 ligands, were stimulated with B3501 and Cap67. Whole *C. neoformans* yeast cells (B3501 and Cap67) did not induce GFP expression by the reporter cells, whereas heat-killed *Candida albicans* (HKCA) used as a positive control showed this activity. The disrupted lysates of B3501 and Cap67 induced GFP expression at equivalent levels to HKCA. These data suggest that *C. neoformans* capsule may not be required for Dectin-2 recognition.

Fig.2 The precipitates of *C.neoformans* lysates induce GFP expression



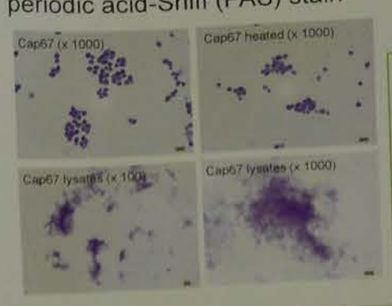
To determine the characteristics of Dectin-2 ligands in *C. neoformans* lysates, the reporter cells were stimulated with untreated or heated lysates, and supernatants or precipitates of B3501 lysates. GFP-inducing activity was detected in heated lysates and in the precipitates, but was not in the supernatants of these lysates. This data suggests that Dectin-2 ligands in *C. neoformans* lysates are heat-tolerant and insoluble materials.

Fig.3 Blocking of GFP expression by H_5IO_4



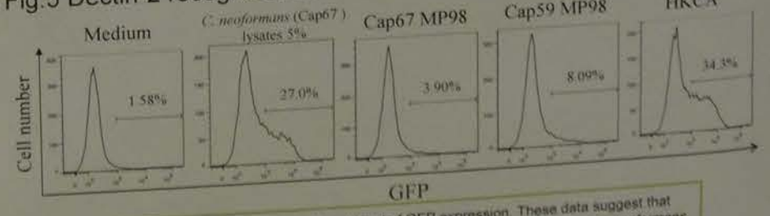
To confirm whether this activity depends on some saccharides, the influences of periodic acid (H_5IO_4) were analyzed in this assay. The lysates-induced GFP expression was strongly inhibited by treatment with H_5IO_4.

Fig.4 *C.neoformans* lysates with periodic acid-Schiff (PAS) stain



These PAS stains show that *C. neoformans* (Cap67) lysates are rich in polysaccharides. Fig. 3 and Fig.4 indicate that Dectin-2 may recognize a kind of saccharides in *C. neoformans*.

Fig.5 Dectin-2 recognizes some mannoproteins in *C.neoformans*



MP98 caused a low, but significant level of GFP expression. These data suggest that mannoprotein such as MP98 could be a candidate of Dectin-2 ligands in *C. neoformans*.

Discussion

These data suggest the presence of some putative Dectin-2 ligands in *C. neoformans*, which may not be expressed on the surface, but rather inside. Capsule may not be required for this activity, because the acapsular mutant strain was as active as the capsular strain. Mannoprotein such as MP98 could be a candidate of Dectin-2 ligands in *C. neoformans*. Further investigation is necessary to define this structure.

Acknowledgement

This work was supported by JSPS KAKENHI Grant Number 15H04867, 16H00643.

科研費 KAKENHI

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ABSTRACT (Revised)

MATERIALS and METHODS

- Organisms tested shown in Table. 1
- Bacterial identification
  MALDI Biotyper (Bruker Daltonics inc.,Inc)
- Antimicrobial susceptibility testing
  DPS1923X (EIKEN CHEMICAL CO., LTD., TOKYO, JAPAN)
  Susceptible results were interpreted using following clinical break point of the clinical laboratory standard institute (CLSI) M100-S23.
- β lacatamase characterization
  Phenotypic and molecular methods
  ESBLs: clavulanic acid mediated inhibition
  AmpC: boronic acid mediated inhibition
  Carbapenemase: modified Hodge test and PCR

Study Protocol

1. Make 0.5 McFarland suspension of organism from overnight isolate on Mueller Hinton Agar
2. Inoculate 25µl organism suspension and 12ml Mueller Hinton Broth into antimicrobial susceptibility test plate and load on DPS1923X (See Figure 2.)
3. Monitor for bacterial growth every hour using kinetic function (See Figure 4.)
4. Calculate the time course of resistance rate to cefpodoxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, aztreonam, imipenem and meropenem. Susceptibility results were interpreted using following clinical break point of the clinical laboratory standard institute (CLSI) M100-S23.

We have shown precise study protocols on Figure 1.

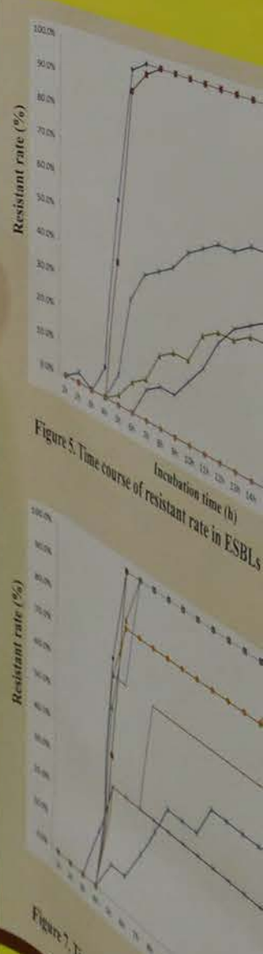


Figure 5. Time course of resistant rate in ESBLs

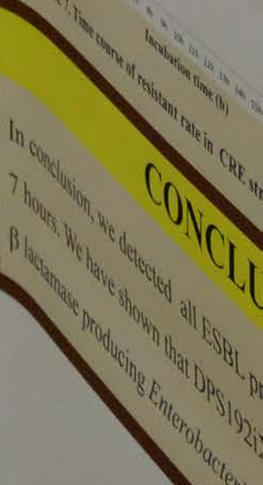


Figure 7. Time course of resistant rate in CRE str

CONCLU

In conclusion, we detected all ESBL-pr
7 hours. We have shown that DPS1923
β lactamase producing Enterobacteriac

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Rapid detection of Multi-Drug Resistant *Enterobacteriaceae* using a new antimicrobial susceptibility test assay

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ABSTRACT (Revised)

BACKGROUND

Recently, the spread of extended spectrum β-lactamase (ESBL) producing bacteria and carbapenem resistant *Enterobacteriaceae* (CRE) in the community has become a problem. A new commercial automated antimicrobial susceptibility test assay, called DPS192iX (EIKEN CHEMICAL CO., LTD., TOKYO, JAPAN) has kinetic system which records bacterial growth every hour. So DPS192iX was expected to contribute to rapid detection of antimicrobial resistance. We evaluated the performance of DPS192iX for rapid detection of β lactamase producing *Enterobacteriaceae*.

Methods

The strains used for analysis were 62 isolates of gram negative *Enterobacteriaceae* including 35 ESBL producing bacteria, 10 AmpC producing bacteria, 7 CRE and 10 negative controls. The drugs used included cefpodoxime (CPDX), ceftazidime, ceftriaxone, cefepime, cefmetazole, aztreonam, imipenem (IPM) and meropenem (MEPM). We recorded bacterial growth hourly and calculated the resistant rates to these drugs.

Results

ESBL producing bacteria, AmpC producing bacteria and CRE presented a high resistant rates to CPDX in 6 hours (ESBL: 97.1%, AmpC: 50.0%, CRE: 100%). Whereas, all negative control strains presented susceptible to CPDX. In addition, CRE were resistant to IMP and MEPM (IPM: 66.7%, MEPM: 33.3%) in 6 hours. On the other hands, all other strains except CRE were susceptible to IPM and MEPM.

Conclusion

In conclusion, we have shown that DPS192iX may contribute to rapid detection of ESBL producing organisms and CRE in 6 hours.

MATERIALS and METHODS

- **Organisms tested shown in Table. 1**
- **Bacterial identification**
  MALDI Biotyper (Bruker Daltonics co.inc)
- **Antimicrobial susceptibility testing**
  DPS192iX (EIKEN CHEMICAL CO., LTD., TOKYO, JAPAN)
  Susceptibility results were interpreted using following clinical break point of the clinical laboratory standard institute (CLSI) M100-S23.
- **β lacatamase characterization**
  Phenotypic and molecular methods
  ESBLs: clavulanic acid mediated inhibition
  AmpC: boronic acid mediated inhibition
  Carbapenemase: modified Hodge test and PCR

Study Protocol

1.Make 0.5 McFarland suspension of organism from overnight isolate on Muellar Hinton Agar

2. Inoculate 25μl organism suspension and 12ml Muellar Hinton Broth into antimicrobial susceptibility test plate and load on DPS192iX® (See Figure 2,3)

3. Monitor for bacterial growth every hour using kinetic function (See Figure 4)

4. Calculate the time course of resistance rate to cefpodoxime, ceftazidime, ceftriaxone, cefepime, cefmetazole, aztreonam, imipenem and meropenem
Susceptibility results were interpreted using following clinical break point of the clinical laboratory standard institute (CLSI) M100-S23.

We have shown precise study protocols on Figure 1.

DATA

Table.1: Organisms tested

Strains	n
ESBLs producing *Enterobacteriaceae*	35
Escherichia coli	27
Klebsiella pneumoniae	8
AmpC β-lactamase producing *Enterobacteriaceae*	10
Enterobacter aerogenes	7
Enterobacter cloacae	2
Escherichia coli	1
Carbapenem resistant *Enterobacteriaceae*	7
*Klebsiella pneumoniae**1	4
*Enterobacter aerogenes**2	2
*Serratia marcescens**2	1
Negative controls*3	10
Escherichia coli	7
Klebsiella pneumoniae	3

*1 *Klebsiella pneumoniae* carbapenemase producing *K. pneumoniae*

*2 Carbapenemase not producing strains

*3 Negative control strains don't produce any β lactamases

Figure 1. Explanation of study protocol

1. Make 0.5 McFarland suspension of organisms

2. Inoculate 25μl organism suspension and 12ml Muellar Hinton Broth into dry plate



Figure.2:
Antimicrobial susceptibility testing plate

3. Load on DPS192iX and implement antimicrobial susceptibility test



Figure.3:
DPS192iX

4. Monitor for bacterial growth every hour and calculate resistance rate to antibiotics by using kinetic system (See Figure.4)

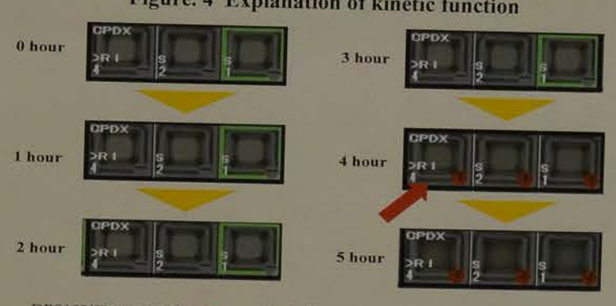


This dry plate has 192 wells per plate

Size
W 65mm×D 85mm×H 15mm
(smaller than iPhone6SE)

Antibiotics
33 to 37 antibiotics can measure

Figure. 4 Explanation of kinetic function



DPS192iX monitored **a decrease in brightness of well** which associated with bacterial growth every hour. If brightness of well is below a specified criteria, this system determines positive for bacterial growth.
In above figure, DPS192iX detected bacterial growth in 4 hour. (Red cursol)

Results

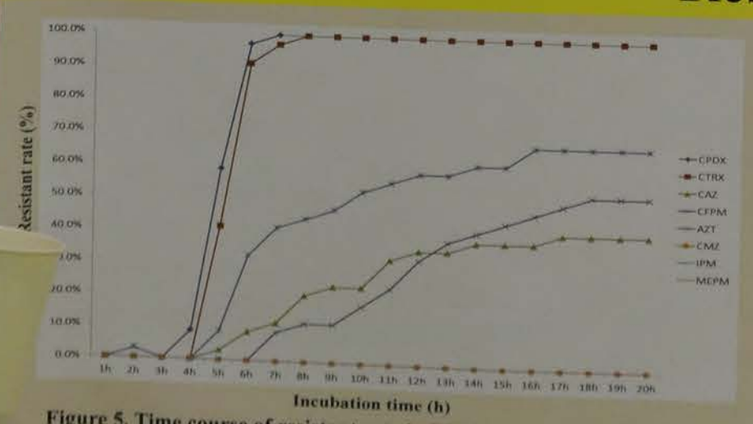


Figure 5. Time course of resistant rate in ESBLs producing strains

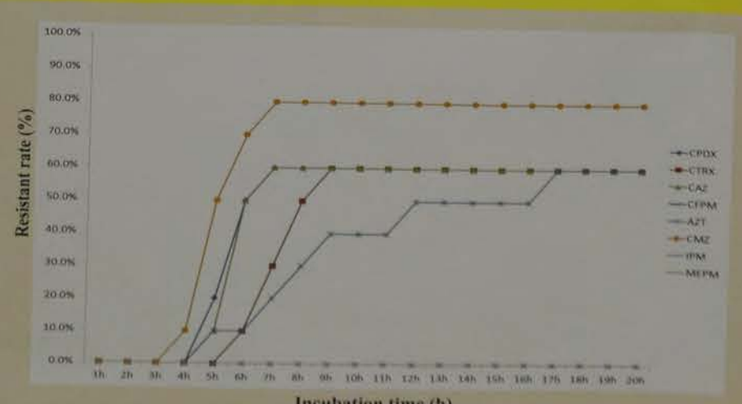


Figure 6. Time course of resistant rate in AmpC producing strains

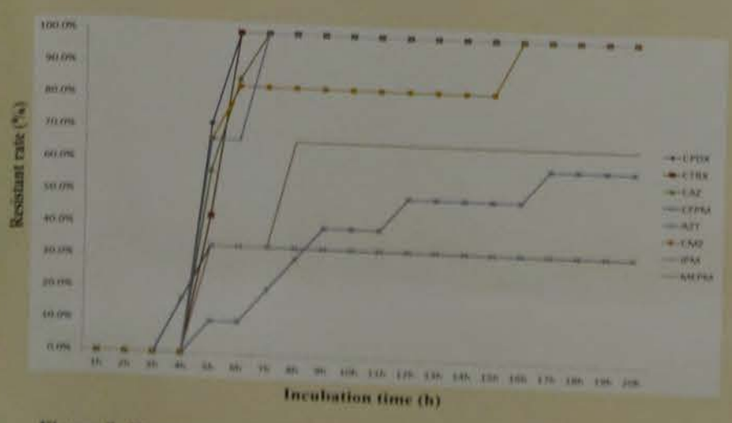


Figure 7. Time course of resistant rate in CRE strains

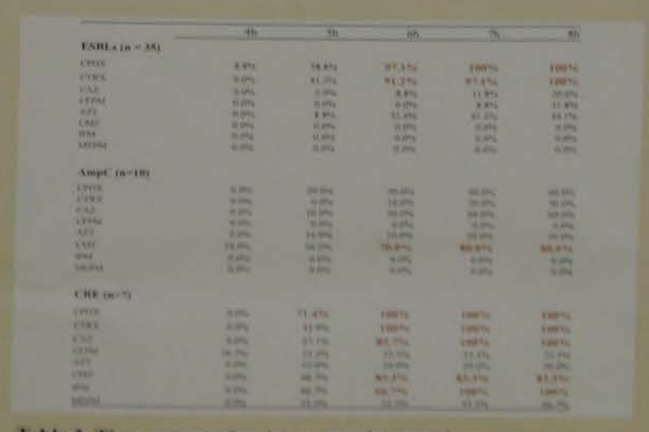


Table 2. Time course of resistant rate in organisms tested

CONCLUSION

In conclusion, we detected all ESBL producing bacteria and CRE in 7 hours. We have shown that DPS192iX contribute to rapid detection of β lactamase producing *Enterobacteriaceae*.

CONTACT'S DETAILS

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Microbiology

PA-75

Comparison of Screening Methods for Carbapenemase-Producing *Enterobacteriaceae*

—Comparison of Modified-Hodge test, Carba NP test, and carbapenem inactivation method—

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Abstract

Background: The global emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) has become a major concern. On the other hand, several laboratory procedures have been developed to detect CPE in a clinical laboratory. In this study, we evaluated the performance of three screening methods, the Modified-Hodge test (MHT), Carba NP test (CNPt), and the carbapenem inactivation method (CIM) for CPE.

Materials and Methods: Twenty three non-carbapenemase producing isolates (Extended-spectrum β-lactamase producers and AmpC producers) and 33 CPE (IMP, 16; NDM, 8; OXA-48 like, 7; KPC, 2) isolates from Japanese hospitals were used in this study. The MICs of imipenem, meropenem, and ertapenem were determined by using the Etest. MHT, CNPt, and CIM were compared as screening methods for CPE. CIM was performed with three carbapenem disks (imipenem, meropenem, and ertapenem).

Results: MIC ranges of imipenem, meropenem, and ertapenem for CPE were 0.5–>32 μg/mL, 0.25–>32 μg/mL, and 0.25–>32 μg/mL, respectively. The sensitivities of MHT (ertapenem), CNPt, and CIM (meropenem) were 90.9%, 87.9%, and 97.0%, respectively, and their specificities were 65.0%, 100%, and 95.7%, respectively. Although false-negative results were observed in OXA-48 like producers and/or mucoid NDM producer by using MHT and/or CNPt, CIM showed positive results in these isolates. When the IPM disk was used in CIM, false-positive results were particularly observed in AmpC over-producers.

Conclusion: Although CIM requires time for cultivation, it showed excellent specificity, sensitivity, and cost-effectiveness. Therefore, CIM is useful in general clinical laboratories. Furthermore, our results indicate that in CIM, meropenem or ertapenem disks should be used instead of imipenem disks. Since CNPt results were obtained more rapidly compared to the other two methods, CNPt might be useful for the rapid prevention and control of infection in a hospital.

Background

Since carbapenems have a broad antibacterial spectrum, these antibiotics are the last resort for controlling infections caused by Gram-negative bacteria.

However, recently, carbapenemase-producing *Enterobacteriaceae* (CPE) that inactivate these antibiotics have increased and have emerged worldwide and become a major concern (Patel et al., 2013). Because CPE have multi-drug resistance, it is often difficult to treat infections caused by these bacteria using antibiotics.

CPE are important pathogens known to cause hospital infections; therefore, more rapid and highly sensitive detection methods for CPE in clinical laboratories are critical for rapid infection prevention and control.

Objective

Although there are several CPE screening methods, there is not sufficient available information for CPE screening methods.

Therefore, we evaluated the performance of three screening methods, Modified-Hodge test, Carba NP test, and carbapenem inactivation method for CPE.

Methods

■ Bacterial strains

Fifty six isolates were tested in this study. Among 56 isolates, the 23 non-carbapenemase producing isolates (Extended-spectrum β-lactamase producers and AmpC producers) and 33 carbapenemase producing isolates (IMP; 16, NDM; 8, OXA-48 like; 7, KPC; 2) isolated from Japanese hospitals were used (Table 1).

■ Antimicrobial susceptibility testing

The MICs of imipenem, meropenem, and ertapenem were determined using the Etest.

■ Molecular carbapenemase detection

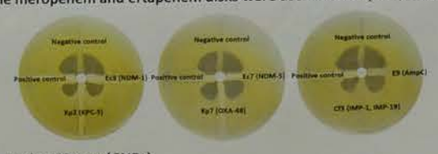
The presence of bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and $bla_{OXA\text{-}48\text{-}like}$ was confirmed using previously published primer sets (Dallenne et al., 2010; Szabó et al., 2005; Notake et al., 2013). Purified PCR fragments were sequenced and sequences used in a similarity search with the BLAST program (DDBJ, Shizuoka, Japan).

■ Phenotypic carbapenemase detection

Klebsiella pneumoniae ATCC BAA-1705 and ATCC BAA-1706 were used as a positive and negative control, respectively.

1. Modified-Hodge test (MHT)

The meropenem and ertapenem disks were used in MHT (CLSI, 2015).



2. Carba NP test (CNPt)

The CNPt was performed by according to the original method (Nordmann et al., 2012) with some modification; the concentration of imipenem was 6 mg/mL and the ratio of bacterial lysate and substrate liquid was 1: 1 (50 μL each).



3. Carbapenem inactivation method (CIM)

The CIM (van der Zwaluw et al., 2015) was performed using imipenem, meropenem, and ertapenem disks with overnight cultivation. When the inhibitory diameter was less than 20 mm using meropenem disk, the results of the CIM were judged as positive.



Results

Table 1. Comparison of screening methods for CPE, and MICs of carbapenem antibiotics.

	Species / Strain no.	Carbapenemase genes[d]	Other *bla*genes: TEM	SHV	CTX-M	pAmpC	MICs (mg/L): Imi-penem	Mero-penem	Erta-penem	Screening methods for CPE[a]: CIM (mm): Imi-penem	Mero-penem	Erta-penem	CIM[b] decision	MHT: Mero-penem	Erta-penem	CNPt
Class A	*Klebsiella pneumoniae* Kp1	KPC-2		SHV-11	CTX-M-15		32	8	16	0	0	0	+	+	+	+
	Kp2	KPC-3	TEM-1	SHV-11			>32	>32	>32	0	0	0	+	+	+	+
	Citrobacter freundii Cf1	IMP-1		SHV-12	CTX-M-9		2	2	6	0	26	28	-	-	-	-
	Cf2	IMP-1					4	8	4	0	0	0	+	+	+	+
	Cf3	IMP-1, IMP-19					8	2	2	0	0	0	+	+	+	+
	Enterobacter cloacae E1	IMP-1					16	4	2	0	0	0	+	+	+	+
	E2	IMP-1		SHV-12	CTX-M-9		>32	>32	>32	0	0	0	+	+	+	+
	E3	IMP-1					16	4	4	0	0	0	+	+	+	+
	E4	IMP-1					4	1	1	0	0	0	+	+	+	+
	E5	IMP-19					4	2	2	0	0	0	+	+	+	+
	E6	IMP-19					8	2	4	0	0	0	+	+	+	+
	E7	IMP-19					2	0.5	1	0	0	0	+	+	+	+
	Escherichia coli Ec1	IMP-1					2	1	2	0	0	0	+	+	+	+
	Ec2	IMP-19					4	0.5	1	0	0	0	+	+	+	+
Class B	Ec3[c]	NDM-1	TEM-1				16	8	8	0	0	0	+	-	+/-	+/-
	Ec4	NDM-1					16	8	8	0	0	0	+	-	+/-	+
	Ec5	NDM-1			CTX-M-15	CMY-42	>32	>32	>32	0	0	0	+	-	+	+
	Ec6	NDM-5					>32	>32	>32	0	0	0	+	+	+	+
	Ec7	NDM-5	TEM-1			CMY-42	>32	32	>32	0	0	0	+	+	+	+
	Ec8	NDM-5				CMY-42	>32	>32	>32	0	0	0	+	+	+	+
	Klebsiella oxytoca K1	IMP-1					16	2	4	0	0	0	+	+	+	+
	Klebsiella pneumoniae Kp3	IMP-19		SHV-1			4	1	2	0	0	0	+	+	+	+
	Kp4	IMP-19		SHV-27			0.5	0.25	0.25	0	0	0	+	+	+	+
	Kp5[c]	NDM-1		SHV-1			>32	16	8	0	0	0	+	-	-	+
	Kp6	NDM-1	TEM-1	SHV-11	CTX-M-15	CMY-6	>32	>32	>32	0	0	0	+	+/-	+	+
	Serratia marcescens S1	IMP-11	TEM-1		CTX-M-9	CMY-48	4	1	1	0	0	0	+	+	+	+
	Escherichia coli Ec9	OXA-48	TEM-1		CTX-M-55		4	1	4	0	0	0	+	+	+	+
	Klebsiella pneumoniae Kp7	OXA-48	TEM-1		CTX-M-15		>32	>32	>32	0	0	0	+	+	+	-
Class D	Kp8	OXA-48					2	0.5	0.5	0	0	0	+	+	+	+
	Kp9	OXA-48	TEM-1		CTX-M-15		2	0.25	0.5	0	0	0	+	+	+	+
	Kp10	OXA-48					8	0.5	1	0	0	0	+	+	+	+
	Kp11	OXA-181, NDM-1	TEM-1		CTX-M-15	CMY-4	16	>32	>32	0	0	0	+	+	+	+/-
	Kp12	OXA-181	TEM-1		CTX-M-15		16	>32	>32	0	0	23	+	+	+	-
non CPE	*Citrobacter freundii* Cf4	ND[d]					0.032	0.5	0.125	0	28	26	-	+/-	+	-
	Enterobacter aerogenes Ea1	ND					>32	>32	>32	0	26	27	-	-	-	-
	Ea2	ND					>32	16	>32	0	26	28	-	-	-	-
	Enterobacter cloacae E8	ND			CTX-M-9		>32	16	>32	0	18	26	+	+	+	-
	E9	ND					2	2	8	0	26	27	-	+/-	+	-
	E10	ND					0.064	0.5	0.125	22	28	29	-	-	+/-	-
	Escherichia coli Ec10	ND				CMY-2	0.5	0.125	0.5	0	23	28	-	+/-	+	-
	Ec11	ND				CMY-2	2	0.125	0.5	24	26	28	-	-	-	-
	Ec12	ND		SHV-12			0.25	0.032	0.125	24	26	29	-	-	-	-
	Ec13	ND		SHV-12			0.25	0.032	0.032	22	26	29	-	-	-	-
	Ec14	ND	TEM-1		CTX-M-14		0.5	0.032	0.064	22	25	29	-	-	-	-
	Ec15	ND			CTX-M-15		0.25	0.032	0.016	23	27	29	-	-	-	-
	Ec16	ND			CTX-M-15		0.5	0.032	0.032	23	27	29	-	-	-	-
	Ec17	ND	TEM-1		CTX-M-14	CMY-2	0.5	0.032	0.064	21	26	28	-	-	-	-
	Ec18	ND			CTX-M-2		0.5	0.064	0.125	0	23	27	-	-	+/-	-
	Ec19	ND			CTX-M-15		0.032	0.5	0.012	23	28	28	-	-	+/-	-
	Klebsiella pneumoniae Kp13	ND				DHA-1	>32	4	32	0	24	27	-	-	-	-
	Kp14	ND				DHA-1	0.5	0.064	0.25	0	26	28	-	-	-	-
	Kp15	ND	TEM-1		CTX-M-3		0.5	0.064	0.032	23	26	29	-	-	-	-
	Kp16	ND	TEM-1	SHV-1	CTX-M-14		0.25	0.032	0.125	23	27	29	-	-	-	-
	Kp17	ND	TEM-1	SHV-26			0.032	0.5	0.012	23	28	29	-	-	-	-
	Proteus mirabilis P1	ND			CTX-M-2		1	0.064	0.016	22	26	28	-	-	-	-
	Serratia marcescens S2	ND	TEM-1				4	4	16	0	27	26	-	-	+/-	-

[a] False-positive results and false-negative results were shown in highlight.
[b] CIM decision was used by meropenem disk (positive, inhibition diameter; < 20 mm)
[c] Mucoid isolates
[d] ND; Not detected

The ertapenem disk yielded more specific results than the other two disks in the CIM. However, the inhibitory diameter with the ertapenem disks in the CIM was observed for the OXA-181 producer.

CIM had high sensitivity and specificity for CPE detection, and that it was able to simplify a positive judgment, unlike with CNPt and MHT.

Table 2. Sensitivity and specificity of each screening method.

	CIM			MHT		CNPt
	Imipenem	Meropenem	Ertapenem	Meropenem	Ertapenem	
Sensitivity (%) [a]	100	97.0	93.9	81.8	90.9	87.9
Specificity (%) [a]	56.5	95.7	100	82.6	65.0	100

[a] In calculation of sensitivity and specificity, +/- results were calculated with positive.

Discussion & Conclusion

- One limitation of the study is that we did not have a sufficient number of isolates including various genotypes such as Ambler class A CPE (GES, SME, IMI, etc.). Further studies using the CIM are necessary for the detection of various types of CPE.
- Although the CIM requires cultivation time, it shows excellent specificity and sensitivity and is inexpensive. In addition, the results of the CIM are not as obscure as those of the other two methods, and the CIM does not use specialized equipment. Therefore, we believe that the CIM will be useful in a general clinical laboratory.
- Furthermore, our results indicate that meropenem or ertapenem disks, and not imipenem disks, should be used in the CIM.
- Since the results of the CNPt can be obtained more rapidly compared to those of the other two methods, the CNPt might be useful for the rapid prevention and control of infections in a hospital.
- The CIM compared with MHT is a more rapid test and it can help simplify positive judgment unlike the MHT; therefore, the CIM may be a more useful laboratory procedure than the MHT.

We believe that our data will be valuable for routine diagnostic tests of CPE in the future.

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Microbiology

PA-76

Microbiology

PA-76

False-nagative rate in VersaTREK blood culture system

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Introduction

- The VersaTREK (VT) (Thermo Scientific) has been introduced as an automated blood culture system in our hospital.
- The VT REDOX1 EZ Draw 40 mL (aerobic) and VT REDOX2 EZ Draw 40 mL (anaerobic) are used as culture bottles. Inoculated culture bottles are incubated until they are determined as positive or are incubated for 7 days.
- Detection of positive culture by the VT system is based on measuring pressure changes in the bottle headspace (due to consumption and/or production of gas by microorganisms) with an external pressure sensor. We can view a graph of the gas pressure by a simple and easy touch panel operation.
- From February to April 2015, when we were viewing graphs of the culture bottles that were determined as negative by the VT system, we observed that some bottles (1 aerobic and 2 anaerobic bottles) showed a non-typical negative graph (**Figure 1**). In these bottles, bacteria were detected by a subculture.
- This study was conducted to analyze the rate of false-negative results (i.e., not detected by the VT system, but positive on terminal subculture) in blood cultures using the VT system.

Methods

- A total of 3228 blood culture bottles (1624 aerobic and 1604 anaerobic bottles) that were submitted to our laboratory from 15 June to 14 December 2015 were analyzed using the VT system.
- At the end of 7 days, negative bottles were removed from the VT system and subcultured.
- **Table 1** shows the agar plates and incubation conditions for subculture. All inoculated agar plates were incubated for 7 days.
- Identification of bacterium and fungus was performed using microscopy, biochemistry tests, and an automatic system, MicroScan WalkAway 40 Plus (Beckman Coulter).

Negative ?

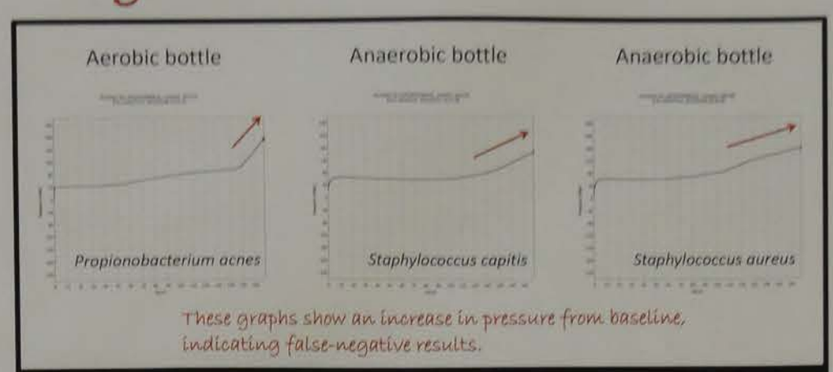


Figure 1. Graphs of gas pressure from three false-negative blood culture bottles.

Table 1. Subculture plate and incubation conditions

Subculture plate	Incubation	
	Temp °C	Atmos
Drigalski agar, Sabouraud dextrose agar	35	aerobic
Sheep blood agar, Chocolate agar	37	5% CO_2
Anaero Columbia agar	37	anaerobic
Anaero Columbia agar	42	microaerobic

Results

- Among the aerobic bottles, 1461 were determined to be negative by the VT system, and no microbial growth occurred by subculture (**Table 2**).
- Among the anaerobic bottles, 1488 were determined to be negative by the VT system. Of these, 11 (0.74%) bottles showed microbial growth on subculture (**Table 2**).
- From 11 bottles, 11 isolates (one isolate per bottle) were detected. These represented 4.0% (11/273) of the total isolates that were detected from aerobic and anaerobic bottles during this study (**Table 3**).
- In these 11 isolates, bottles in which *Campylobacter jejuni* and *Staphylococcus epidermidis* were detected showed a pressure change in gas as shown by graph. However, in bottles in which other microorganisms were detected, there was little or no change in pressure (**Figure 2**).

Table 2. Summary of culture results

VersaTREK system result	No. of bottles determined to be positive or negative by terminal subculture			
	Aerobic bottles		Anaerobic bottles	
	Positive	Negative	Positive	Negative
Positive	149	14	101	15
Negative	0	1461	11	1477

Table 3. Distribution of microbial isolates from blood cultures

Microorganisms	Total no. of isolates	No. of isolates			
		Aerobic bottles		Anaerobic bottles	
		VT positive	VT negative	VT positive	VT negative
Gram-negative aerobes					
Acinetobacter spp.	3	3			
Aeromonas spp.	4	2		2	
Citrobacter spp.	10	5		5	
Enterobacter spp.	10	5		5	
Escherichia coli	62	39		22	1
Klebsiella spp.	29	17		12	
Morganella morganii	2	2			
Pseudomonas aeruginosa	4	3			1
Salmonella sp.	2	1		1	
Serratia sp.	2	1		1	
Campylobacter jejuni	5	2			3
Helicobacter cinaedi	1				1
Gram-positive cocci					
Staphylococcus aureus	7	4		3	
Other staphylococci	18	15		2	1
Enterococcus spp.	27	14		13	
Streptococcus agalactiae	25	15		10	
Streptococcus dysgalactiae	13	7		6	
Other streptococci	21	11		10	
Stomatococcus sp.	1	1			
Gram-positive bacilli					
Bacillus spp.	10	6		4	
Corynebacterium spp.	4	3		1	
Anaerobes					
Bacteroides spp.	4			4	
Prevotella sp.	2			2	
Clostridium sp.	1			1	
Propionibacterium acnes	2			1	1
Parvimonas micra	3			1	2
Fungi					
Aspergillus niger	1				1
Total	273	156	0	106	11

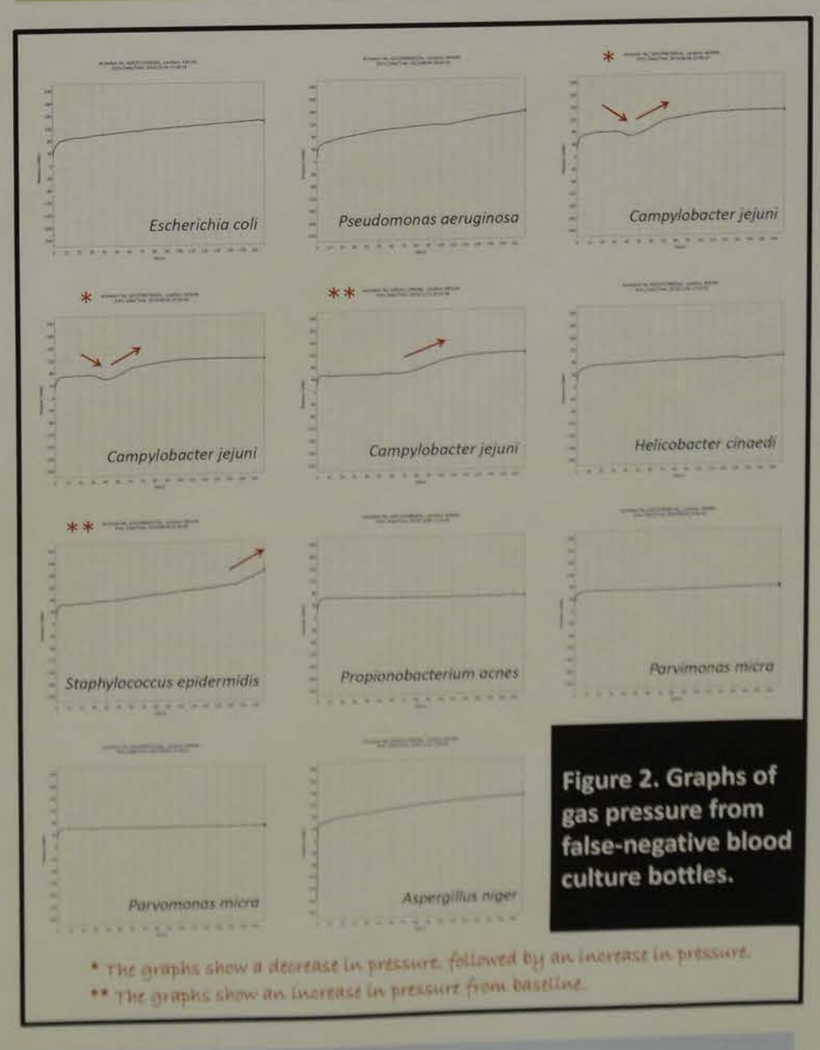


Figure 2. Graphs of gas pressure from false-negative blood culture bottles.

Conclusion

- The false-negative rate in anaerobic bottles was 0.74% in our study. This rate is higher than that of 0.2% shown in a clinical trial performed at seven clinical sites (described in the VT instructions).
- In our study, terminal subculture was performed for all culture bottles that were determined to be negative by the VT system. However, performing routine terminal subculture is difficult because of the cost of labor and labor-intensive practice.
- Some of the false-negative bottles showed a change in pressure in graphs.
- We hope that the computer algorithm for microbial detection in the VT system will be improved in the future.
- At present, we believe that the following procedures will lead to reducing some of the false-negative results. Before discarding the culture bottles that are determined to be negative by VT system, researchers should i) perform a review of the graphs, and ii) perform a subculture from the bottle to show any non-typical negative graphs.

Microbiology

PA-77

Clinical utility of a two-step culture method for the detection of group B *streptococcus* in pregnant women

~ GBS detection rate with the combination of selective separation and enrichment media ~

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Central Clinical Laboratory, Nara Medical University Hospital

Abstract

In this study, we evaluated the performance of a two-step culture method using the combination of selective separation and enrichment media for GBS screening of pregnant women. With this method, GBS was selectively cultured in enrichment media and clearly identified in separation media that distinguishes pale pink to red GBS colonies.

The GBS detection rate was significantly improved [17.7% (130/736)] with the two-step culture method compared to the direct culture method [8.3% (75/903)]. Additionally, a non-ß-hemolytic strain of GBS was detected with the two-step culture method.

These results demonstrate that the combination method is useful in the prevention of neonatal GBS infection by improved GBS detection rate in pregnant women.

Introduction

GBS is a causative bacterium of meningitis and sepsis in neonates. The Centers for Disease Control and Prevention in the United States has recommended GBS screening of all pregnant women at 35-37 weeks of gestation to prevent neonatal GBS infection.

The aim of the present study was to evaluate the performance of a two-step culture method using the combination of selective separation and enrichment media for GBS screening of pregnant women.

Materials and Methods

Vaginal and anorectal clinical swab specimens was taken from each pregnant woman.

903 specimens from March 2014 to March 2015 as the direct culture method, and 736 specimens from April 2015 to February 2016 as the two-step culture method using the combination of selective separation and enrichment media.

(1) Direct culture method

The direct culture method were plated directly on Try/Soy Blood Agar (Sheep) No.2 (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), and were incubated for 24 and 48 hours at 35 ℃. Then colonies were picked and it was identified GBS by additional testing(Figure 1).

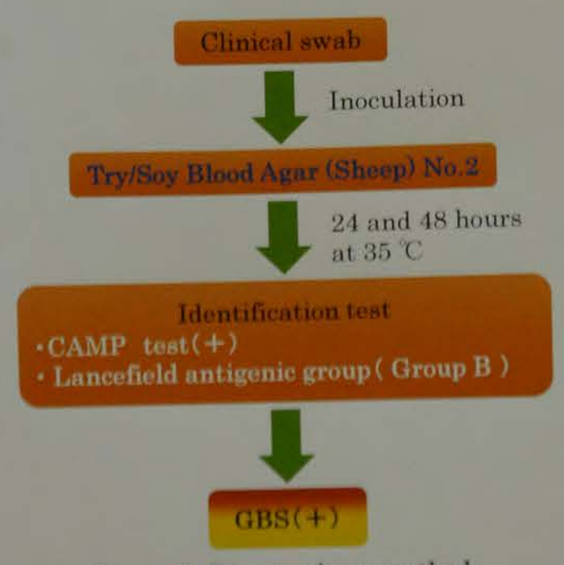


Figure 1: Direct culture method

(2) Two-step culture method

The two-step culture method were cultured a selective enrichment medium (Todd-Hewitt CNA Bouillon, Sysmex bioMérieux Co., Ltd., Tokyo, Japan) , and were incubated for 24 and 48 hours at 35 ℃. The specimens that did not growth was determined as GBS negative. When the selective enrichment medium was showed growth, one loopful (10µl) of the bacterial solution was subculture to a selective separation medium (chromID StreptoB, Sysmex bioMérieux Co., Ltd., Tokyo, Japan) for 24 and 48 hours at 35 ℃. Then pale pink to red colonies were picked and it was identified GBS by additional testing(Figure 2).

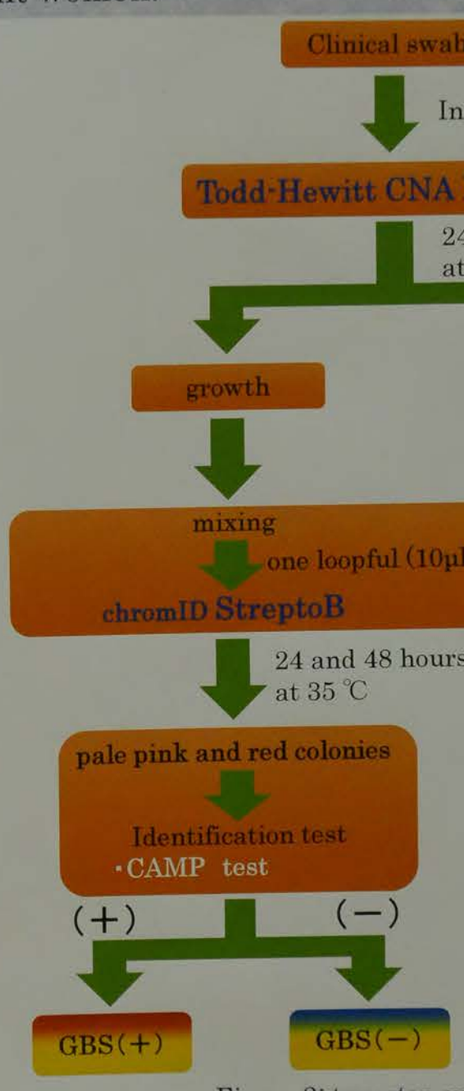


Figure 2: two-step culture method

Results

The GBS detection rate was 8.3% (75/903) with the direct culture method and 17.7% (130/736) with the two-step culture method(Figure 3).

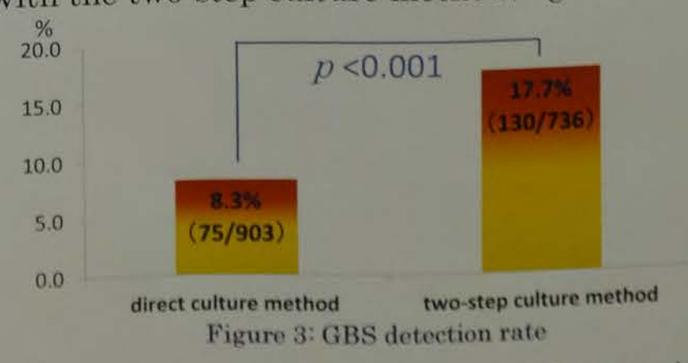


Figure 3: GBS detection rate

Identification of GBS was clear and easy with the two-step culture method that distinguishes pale pink to red GBS colonies. Also, a non-ß-hemolytic strain of GBS was detected same color colonies with the two-step culture method(Figure 4).



Figure 4: GBS colonies
left:Try/Soy Blood Agar (Sheep) No.2, right:chromID StreptoB

Conclusion

These results demonstrate that the combination method using selective separation and enrichment media was more sensitive than the direct culture method for GBS screening of pregnant women, indicating its usefulness in the prevention of neonatal GBS infection.

Microbiology

PA-78



Evaluation of urine flow cytometer Sysmex UF-5000®: the comparison with routine methods for identifying bacteria

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2) Department of Infectious Diseases, Kanazawa Medical University, Japan

Correction

The content is different from the abstract because the data were reanalyzed by the latest UF-5000 algorithm.

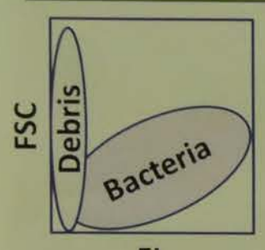
Purpose

To evaluate the performance of a novel flow cytometer-based analyzer, Sysmex UF-5000 (UF-5000) for classifying bacteria by comparing with gram staining and culture identification results.

Principle of UF-5000 for classifying bacteria

UF-5000 can classify bacteria from urine samples together with other urine particles within a few minutes. It adopts the new staining reagents and blue laser, thereby improving the classification accuracy than the previous model "UF-1000i".

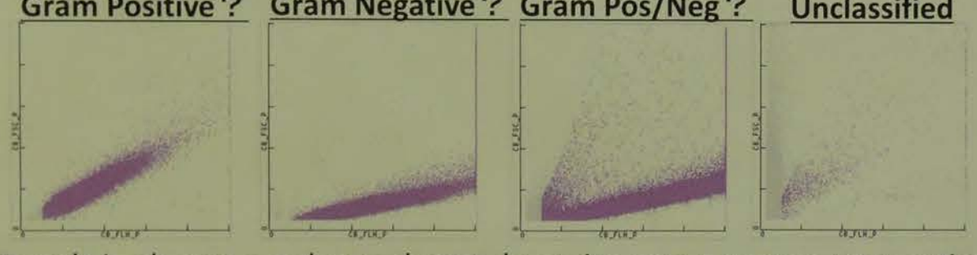
UF-5000 bacteria scattergram



X axis: FL (Side Fluorescence Intensity)
Y axis: FSC (Forward Scatter Light Intensity)

- "FL" provides information on nucleic acids contents of bacteria.
- "FSC" provides information on the size.

Gram Positive？ **Gram Negative？** **Gram Pos/Neg？** **Unclassified**



Result is shown as above depends on its convergence properties. And it is reported in either of four patterns as shown above by the internal algorithm analysis.

Materials and methods

- **Period:** Jun.01.2015 - Jan.31.2016
- **Site:** Kanazawa Medical University Hospital
- **Objective:** Urinary tract infection-suspected urine sample

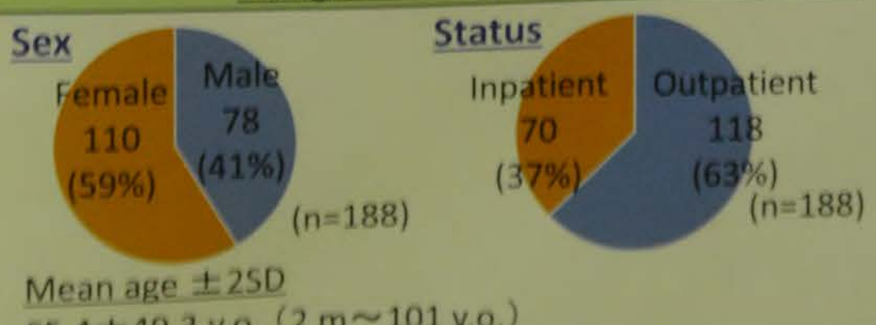
Inclusion Criteria:

✓ Gram positive and/or negative bacteria were confirmed in Gram staining with direct smear examination.
✓ ≥10 white blood cells/μL and ≥100 bacteria/μL in UF-5000 measurement.

- The results of UF-5000 were compared with Gram staining and culture identification.
- Concordance rate (CR) and positive predictive value (PPV) were calculated.

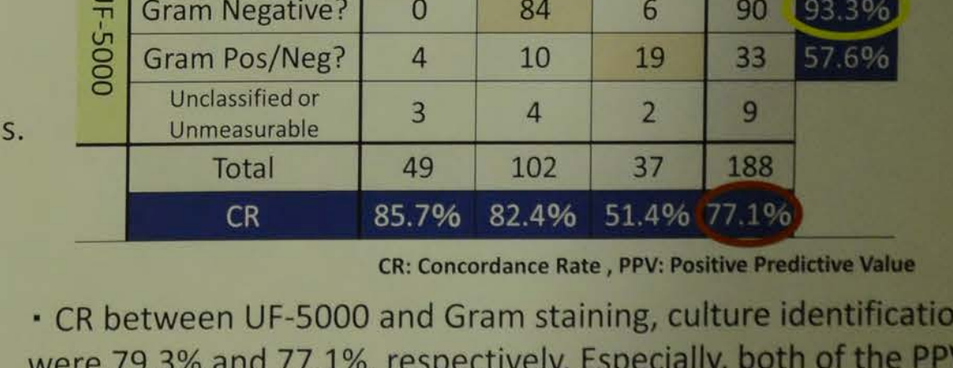
Results

Backgrounds of patients



Mean age ±2SD
65.4±49.3 y.o. (2 m～101 y.o.)

Isolated microorganisms



Comparisons with the conventional methods

		Gram staining				
		Gram Positive	Gram Negative	Gram Pos/Neg	Total	PPV
UF-5000	Gram Positive?	42	8	6	56	75.0%
	Gram Negative?	1	84	5	90	93.3%
	Gram Pos/Neg?	4	6	23	33	69.7%
	Unclassified or Unmeasurable	2	5	2	9	
	Total	49	103	36	188	
	CR	85.7%	81.6%	63.9%	79.3%	

CR: Concordance Rate , PPV: Positive Predictive Value

		Culture identification				
		Gram Positive	Gram Negative	Gram Pos/Neg	Total	PPV
UF-5000	Gram Positive?	42	4	10	56	75.0%
	Gram Negative?	0	84	6	90	93.3%
	Gram Pos/Neg?	4	10	19	33	57.6%
	Unclassified or Unmeasurable	3	4	2	9	
	Total	49	102	37	188	
	CR	85.7%	82.4%	51.4%	77.1%	

CR: Concordance Rate , PPV: Positive Predictive Value

- CR between UF-5000 and Gram staining, culture identification were 79.3% and 77.1%, respectively. Especially, both of the PPVs were high (93.3%) when UF-5000 classified as "Gram negative?".

Mismatch cases

UF-5000	Match	Mismatch	Match	Match	Mismatch
Gram staining	Match	Match	Mismatch	Match	Mismatch
Culture identification	Match	Match	Match	Mismatch	Mismatch
Number (n=188)	135 71.8%	25 13.3%	10 5.3%	14 7.4%	4 2.1%

4 of 25 cases looked like "Gram negative?" on the scattergrams as shown below.



UF-5000	Unclassified	Gram Pos/Neg?	Gram Pos/Neg?	Gram Positive?
Gram staining / Culture identification	Gram Negative?			

- 135 (71.8%) cases were fully matched in the 3 methods, however, UF-5000 results were different from the other 2 methods in 25 cases (13.3%) . The above 4 cases can be expected to match by the algorithm improvement .

Conclusion

UF-5000 can potentially provide rapid classification of bacteria compared with the Gram stain and culture identification in urine samples within a few minutes. It has limits of analytical abilities compared with the conventional methods, however, updating the analytical algorithm may improve its performance.

Microbiology

PA-79

Bacteremia Caused by *Capnocytophaga* Species in Kyushu University Hospital, 2005-2015

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Department of Clinical Chemistry and Laboratory Medicine[1], Center for the Study of Global Infection[2], Department of Pediatrics[3], Department of Clinical Immunology and Rheumatology/Infectious Disease[4], Department of Clinical Chemistry and Laboratory Medicine Graduate School of Medical Sciences[5], Kyushu University Hospital, Fukuoka, Japan

【Background】

The genus *Capnocytophaga* is a group of facultative, gram negative rod-shaped anaerobe. These anaerobes constitute a fraction of normal oral flora in humans and animals. It has been reported that these anaerobes are predominantly present in the oral cavity from childhood. Bacteremia caused by *Capnocytophaga* species is generally rare, but it is known that it occurs in immunocompromised hosts. *Capnocytophaga canimorsus* is known to cause serious zoonotic infections in recent years. The genus *Capnocytophaga* is known to produce β-lactamase and has been reported to be able to mutate into highly resistant strains.

【Methods】

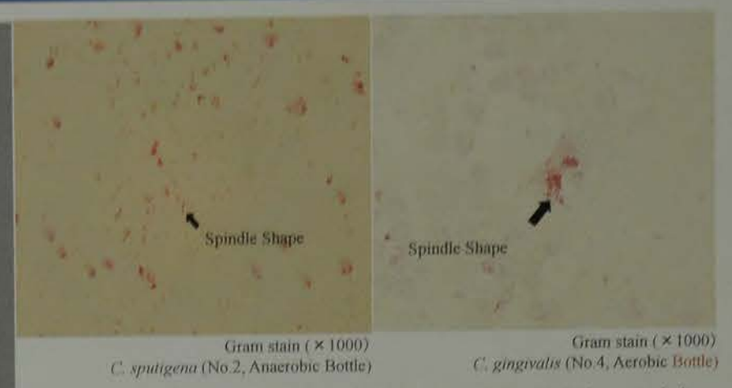
We studied *Capnocytophaga* bacteremia cases in Kyushu University Hospital, retrospectively. There were seven cases in which *Capnocytophaga* species were detected in blood cultures from 2005 to 2015. We analysed the clinical background and microbial background data of these cases.

Clinical Background

Underlying diseases, antimicrobials used before and after bacteremia, etc.
Outcome was judged by clinical course (e.g., the result of blood culture and the decline of fever).

Microbial Background

Detection time, identification, sensitivity, β-lactamase, etc.
As the blood culture instrument, Bact/ALERT 3D(Sysmex Biomerieux) was used from 2005 to Mar 2013 and Bactec FX(BD) was used afterward. The species were identified using ID test HN-20 (Nissui), VITEK MS (Sysmex Biomerieux), and 16SrRNA. For sensitivity test, bacteria were grown on Dry plate Eiken (Eiken) with 5% CO_2 supplementation and MIC was determined after 20 hour incubation. Beta lactamase production was tested by the nitrocephine method and whole genome sequencing of beta lactamase was analyzed.



Gram stain (×1000) *C. sputigena* (No.2, Anaerobic Bottle)

Gram stain (×1000) *C. gingivalis* (No.4, Aerobic Bottle)

Figure 2. A gram stain from the positive blood culture bottle. A characteristic spindle shaped organism is shown.



Capnocytophaga sputigena *Capnocytophaga gingivalis*

Figure 3. A colony of *Capnocytophaga sputigena* and *Capnocytophaga gingivalis* (5% carbon dioxide culture of 24-48 hours)

【Results】

On these seven cases, clinical background and microorganism data were summarized in Table 1 and Table 2, respectively. These patients had been hospitalized in three clinical departments: four in pediatrics, one in surgery, and two in hematology. The gender ratio was 6 to 1, with males being predominant. All cases suffered bacteremia of *Capnocytophaga* species during chemotherapy for underlying diseases. For febrile neutropenia in chemotherapy, antibiotics have been used. The antibiotics used before bacteremia were meropenem, cefazolin, cefpirome and cefepime. Piperacillin/tazobactam, ciprofloxacin, doripenem and meropenem were used for the treatment after diagnosis of bacteremia.

Clinical Background

Table 1. Seven cases of bacteremia associated with *Capnocytophaga* species.-including present illness and antimicrobial treatment date used before and after becteremia.

Case No.	Date of Admission	Patient's Age (Y/M)	Gender	Department	Inpatient/ Outpatient	Present Illness (PI)	Cause Organism of Bacteremia	Antimicrobials Used Before and After Bacteremia		Outcome
								Before	After	
1	Oct. 2006	21y5m	F	surgery	inpatient	**Low-birth-weight infant under NICU management** History of frequent blood transfusion Decompensated cirrhosis and hepatopulmonary syndrome due to HCV infection Hospitalized for liver transplantation	*C. sputigena*	MEPM MCFG TEIC	P/T CPFX TEIC MCFG	recovered
2	Nov. 2008 (change department)	6y1m	M	pediatrics	inpatient	**Soft tissue tumor in his right(or left) lower leg** Hospitalized in orthopedics and transfer department to pediatrics Under the first round of chemotherapy	*C. sputigena*	MCFG CPP PAPM/BP		recovered
3	Apr. 2011	8y0m	M	pediatrics	inpatient	**Mature B cell leukemia** Prominent myelosuppression after multidrug chemotherapy Administration of G-CSF and γ-globulin for FN	*C. gingivalis*	CPR PAPM/BP		recovered
4	Jul. 2011	53y9m	M	hematology	inpatient	**Malignant lymphoma** A retroperitoneal tumor and multiple intraabdominal lymph nodes enlargements Refractory to the treatment and hospitalized for autologous peripheral blood stem cell transplantation	*C. gingivalis*	CFPM DRPM		recovered
5	Oct. 2014	2y11m	M	pediatrics	inpatient	**Adrenal medulla neuroblastoma** Under the fourth round of chemotherapy Administration of G-CSF for FN and elevation of procalcitonin	*C. sputigena*	CFPM	MEPM+TEIC MEPM	recovered
6	Jan. 2015	5y3m	M	pediatrics	inpatient	**Acute T-cell lymphocytic leukemia** FN(grade4) after induction chemotherapy	*C. sputigena*	CFPM	CFPM+ MEPM	recovered
7	Sep. 2015	56y3m	M	hematology	inpatient →outpatient	**Acute myelogenous leukemia (AML)** Chemotherapy for the recurrence of AML after bone marrow transplantation Unable to oral intake because of painful stomatitis	*C. sputigena*	Nothing	DRPM TEIC MCFG	recovered

CFPM: cefepime, CPFX: ciprofloxacin, CPR: cefpirome, DRPM: doripenem, PAPM/BP: panipenem/betamipron, P/T: piperacillin/tazobactam, MCFG: micafungin, MEPM: Meropenem, TEIC: tercoplanin

Microbial Background

Gram stain from positive blood culture bottles showed gram negative spindle rods, which are characteristic for *Capnocytophaga* species (Figure 2). The colonies of *Capnocytophaga* species shows a characteristic 'sliding form' on culture media plates after 24-48 hours incubation (Figure 3). *Capnocytophaga sputigena* and *Capnocytophaga gingivalis* were isolated from five and two patients, respectively. All the isolated strains produced beta lactamase.

Table 2. The Detection Situation of *Capnocytophaga* species. From Blood Cultures

Case No.	Hospital Day	Detection Bottle (Aerobic/Anaerobic)	Detection Set	Time to Positive Bottle (TTP)	Identification	Beta Lactamase Prodocing (Nitrocephine Method)
1	12	Anaerobic bottle	1/1 set	unknown	*C. sputigena*	Positive
2	79	Anaerobic bottle	1/1 set	unknown	*C. sputigena*	Positive
3	16	Aerobic bottle	1/1 set	unknown	*C. gingivalis*	Positive
4	21	Aerobic bottle	1/2 set	unknown	*C. gingivalis*	Positive
5	33	Aerobic/ Anaerobic bottle	1/1 set/ 2/2 set	74.5hr 36.8hr/64.8hr 43.8hr	*C. sputigena*	Positive
6	27	Aerobic/ Anaerobic bottle	1/1 set	41hr/80hr	*C. sputigena*	Positive
7	0	Aerobic bottle	1/1 set	61.2hr	*C. sputigena*	Positive

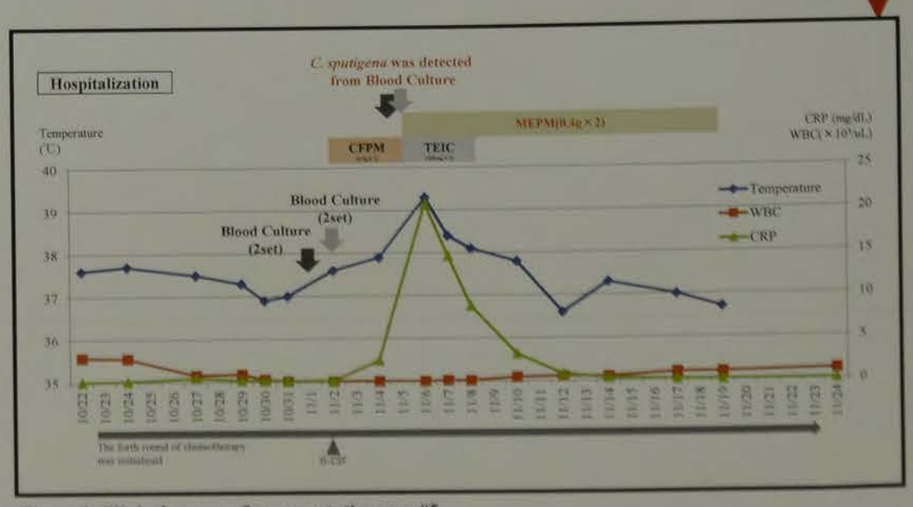


Figure 1. Clinical course of representative case #5
The fourth round of chemotherapy was begun on Oct. 23th. Cefepime was administered for febrile neutropenia, but his fever lasted. The *Capnocytophaga* species continued to be isolated from blood cultures. The antibiotics was changed from cefepime to meropenem and the fever resolved.

Table 3. MIC (μg/ml.) of *Capnocytophaga* species.

	No.1	No.2	No.3	No.4	No.5	No.6	No.7
Identification	*C. sputigena*	*C. sputigena*	*C. gingivalis*	*C. gingivalis*	*C. sputigena*	*C. sputigena*	*C. sputigena*
PCG	1.0	≦8.0	≦8.0	2.0	≦8.0	≦8.0	1.0
ABPC	2.0	≦8.0	≦8.0	2.0	≦8.0	≦8.0	1.0
ABPC/SBT		2.0	≦0.25	≦0.25	≦0.25	2.0	≦0.25
CEZ	≦4.0	≦4.0	1.0	2.0	≦4.0	≦0.5	≦4.0
CTM	≦4.0	≦4.0	1.0	≦4.0	≦4.0	≦4.0	≦4.0
CTRX		≦4.0	2.0	≦4.0	≦4.0	≦4.0	0.25
CTX	0.5						
LMOR	0.25						
CFPM		2.0	≦4.0	≦0.06	1.0	2.0	≦0.06
CCL	16.0	≧32.0	16.0	4.0	≧32.0	≧32.0	8.0
CFPN		≦2.0	1.0	≦2.0	≦2.0	≦2.0	≦0.25
IPM/CS	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06
MEPM	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06
FAM		≦0.12	2.0	≦0.12	8.0	8.0	8.0
AZM		2.0	≦8.0	0.5	≦8.0	≦8.0	≦8.0
CLDM	≧2.0	≦0.12	≦1.0	≦0.12	≦1.0	≦1.0	≦1.0
MINO	≦0.25	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12
LVFX	2.0	≦1.0	≦1.0	≦1.0	≦1.0	≦1.0	4.0
SPFX		≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	1.0
MFLX		≦0.5	≦0.5	≦0.5	≦0.5	≦0.5	1.0
ST		≦80.0	20.0	≦80.0	40.0	80.0	80.0

【Discussion】

The oral mucosa is supposed to be the main portal of entry for *Capnocytophaga* species to cause bacteremia. *Capnocytophaga* bacteremia sometimes occurs in immunocompromised patients, which was observed in our seven cases of this study. It is also more familiar in children rather than in adults, because the flora of the oral cavity in children is different from that in adults.

We usually use broad-spectrum antibiotics for the treatment of febrile neutropenia under chemotherapy. The composition of the oral flora is easily changed as we use these antibiotics. *Capnocytophaga* species becomes dominant as microbial substitution.

Recent article reported a bacteremia case caused by multidrug-resistant *C. sputigena* . This strain possesses ESBL-related *cfx43* gene and resistant to fourth generation cephems, which are usually used for FN as the first-line antibiotic treatment. Considering these cases, we should rapidly identify the species of bacteremia and perform the susceptibility test in order to select appropriate therapeutic agents.

【Conclusion】

During the study period of these 10 years, 7 cases of bacteremia caused by *Capnocytophaga* species were observed in Kyushu University Hospital. All cases were under chemotherapy for underlying diseases. All *Capnocytophaga* species were beta lactamase producing strains and all cases were treated with appropriate antibacterial agents.

Microbiology

PA-80

A case of infectious endocarditis due to *Bartonella quintana*

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Department of Clinical Laboratory, Tenri Hospital

1. Patient profile

A 70-year-old man was admitted to our hospital with intermittent fever lasting for one year.
Specifically, the number of episodes of fever of unknown origin he experienced each month had increased from one or two to approximately 20.

2. Echocardiography





- The vegetation did not exhibit marked oscillation.
- Blood culture-negative endocarditis caused by a low virulence bacillus?

3. Labo data, Immunological tests

RBC	2.41	x10⁴/ul	↓	BUN	20.1	mg/dl	↑	T-Bil	0.5	mg/dl
Hb	7.6	g/dl	↓	CRE	1.4	mg/dl	↑	ALP	164	IU/l
Ht	22.4	%	↓	UA	6.2	mg/dl		CK	207	IU/l ↑
MCV	93	fl		TP	9.1	g/dl	↑	CRP	1.1	mg/dl ↑
Plt	16.4	x10⁴/ul		Alb	3.1	g/dl		PT	12.4	sec
WBC	5.2	x10³/ul		Na	134	mmol/l		PT-INR	1.11	sec
Seg	69.5	%		K	3.7	mmol/l		aPTT	30	sec
Baso	0.8	%		Ca	8.1	mg/dl		D-dimer	11.8	μg/ml ↑
Eosino	0.8	%		LDH	369	IU/l	↑			
Mono	3.4	%	↓	AST	42	IU/l	↑			
Ly	25.5	%		ALT	23	IU/l				

Antinuclear antibody	40	times	↑	PR3-ANCA	316	U/ml	↑
	Speckled	(+)		MPO-ANCA	<1.0	U/ml	
	Nucleola	(+)		ds DNA	18	IU/ml	
IgG	5052	mg/dl	↑	Sm	<7.0	ng/ml	
IgA	261	mg/dl		MMP 3	72.6	ng/ml	
IgM	86	mg/dl		Anti-GBM antibody	88.9	U/ml	↑
CH50	38	U/ml		Fe	25	μg/dl	
C3	82	gm/dl		Sat	13.20%		
C4	10.5	mg/dl	↓	Ferritin	550	ng/ml	↑
Rheumatoid factor	5.6	IU/ml		Haptoglobin	1	mg/dl	↓
Cryoglobulin	(-)			sIL-2R	2012	U/ml	↑

4. Differential diagnosis

① Blood culture(3 set) were all negative

②ANCA-related agitis → Steroid therapy

③Blood culture negative endocarditis(BCNE) → ope

5. Further testing: comprehensively examined the patient's serum antibody titers

Bartonella henselae	titer
IgG	>1024
IgM	20
Bartonella quintana	
IgG	256
IgM	(-)
Chlamydia trachomatis	
IgG	2.7
IgA	0.19
IgM	0.12

Chlamydophila pneumoniae	titer
IgG	1.28
IgA	1.35
IgM	0.35
Brucella	
Abortus	<40
Canis	<160
Candida antigen	<0.02
Aspergillus antibody	0.1
Q fever DNA	(-)
Legionella urine antigen	(-)

Endocarditis due to *Bartonella henselae*?

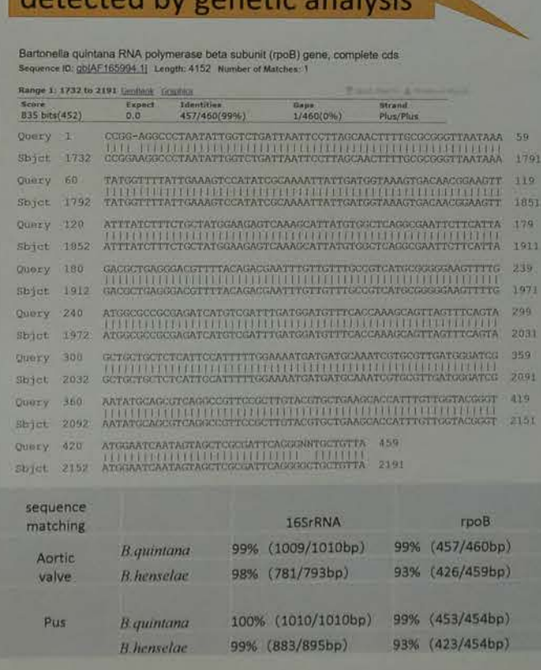
6. Microbiological culture: aortic valve

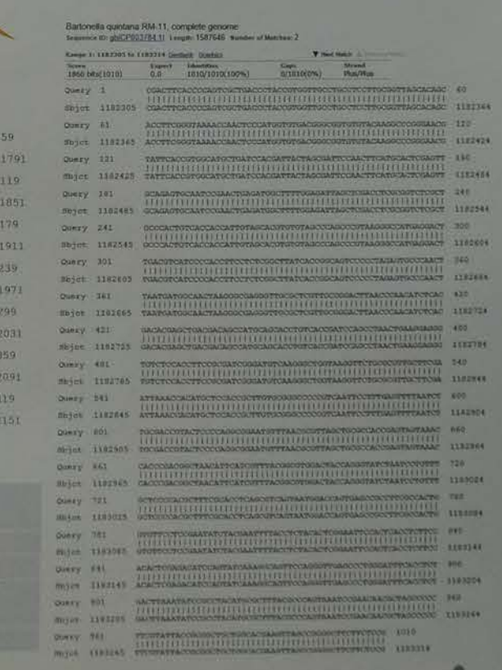


Aortic valve with pus

- After treatment with ceftriaxone, gentamicin, and minocycline, the oscillation of cardiac vegetation increased, then aortic valve replacement was performed.
- 8 weeks and cultured. But the bacteria did not growth
- DNA extruction by Quiagen®(Tissue protocol)

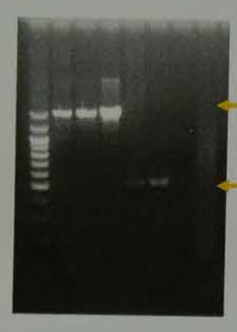
Bartonella quintana was detected by genetic analysis





sequence matching		16SrRNA	rpoB
Aortic valve	*B.quintana*	99% (1009/1010bp)	99% (457/460bp)
	B.henselae	98% (781/793bp)	93% (426/459bp)
Pus	*B.quintana*	100% (1010/1010bp)	99% (453/454bp)
	B.henselae	99% (883/895bp)	93% (423/454bp)

7. DNA sequence of 16SrRNA & rpoB gene



16SrRNA

1500bp

rpoB(500bp)

Nucleotide sequence analysis of the 16S rRNA and rpoB genes were performed after extracting DNA from the aortic valve and pus.

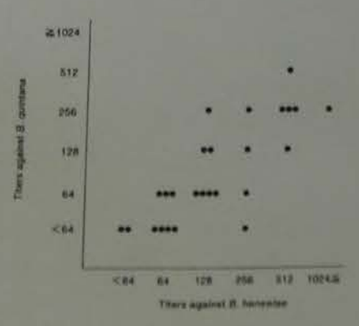
8. Discussion

- We experienced a rare case of infective endocarditis caused by *Bartonella quintana*. *Bartonella* infections are rare, they should be considered in blood culture-negative cases of endocarditis.

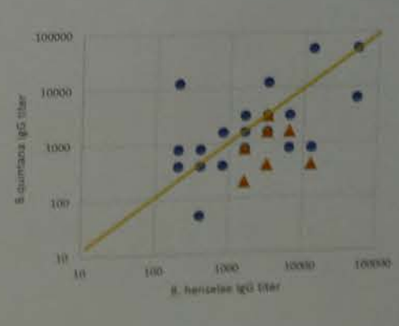
Fournier PE *et al* (Clin Diagn Lab Immunol. 9(4): 795–801, 2002) suggested that the serological tests available at present may not reliably distinguish between antibody responses to Bartonella species. Yamada Y *et al* (Diagn Microbiol Infect Dis. 70(3): 395–398, 2011) reported that patient's who had endocarditis due to *Bartonella* were positive of PR3-ANCA and renal disturbance.

In cases of *Bartonella* infection-induced endocarditis in which it is suspected that cross-reactivity might have occurred during serum antibody titer examinations, genetic analysis is important for discriminating between *Bartonella* sp.

9. Cross-reactivity in *Bartonella* antibodty titer



Tsuneoka H et al: Kansenshogaku zasshi. 74: 387-391, 2000



Fournier PE: Clin Diagn Lab Immunol. 9(4):795-801, 2002

Microbiology

PA-81

A case of pulmonary *Nocardia cyriacigeorgica* infection in a patient with *Mycobacterium intracellulare* lung disease.

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Introduction:*Nocardia* species are Gram-positive, weakly acid-fast, filamentous aerobic bacteria (Figure 1). Pulmonary nocardiosis is commonly recognized as an opportunistic infection in immunocompromised hosts. However, nocardiosis can also affect immunocompetent hosts. In immunocompetent hosts, pulmonary nocardiosis often co-occurs with an underlying disease such as bronchiectasis or chronic obstructive pulmonary disease, or with treatment with steroids. In addition, a few recent reports have described pulmonary nocardiosis with nontuberculosis mycobacterial lung infection [1-4]. Here we report a case of pulmonary *Nocardia cyriacigeorgica* infection in a patient with *Mycobacterium intracellulare* lung disease.

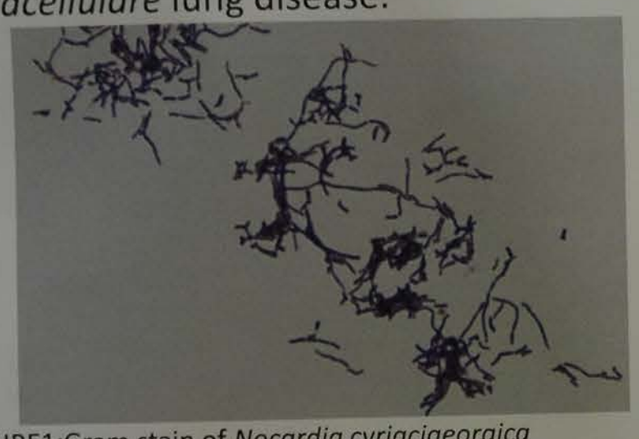


FIGURE1:Gram stain of *Nocardia cyriacigeorgica*

Case presentation: An immunocompetent 79-year-old Japanese woman with untreated *M. intracellulare* lung disease for the past several years was admitted to our hospital because of high fever and cough. Her sputum amount increased and chest X-ray imaging and computed tomography revealed infiltrates in the lower lobe of the right lung (Figure 2).

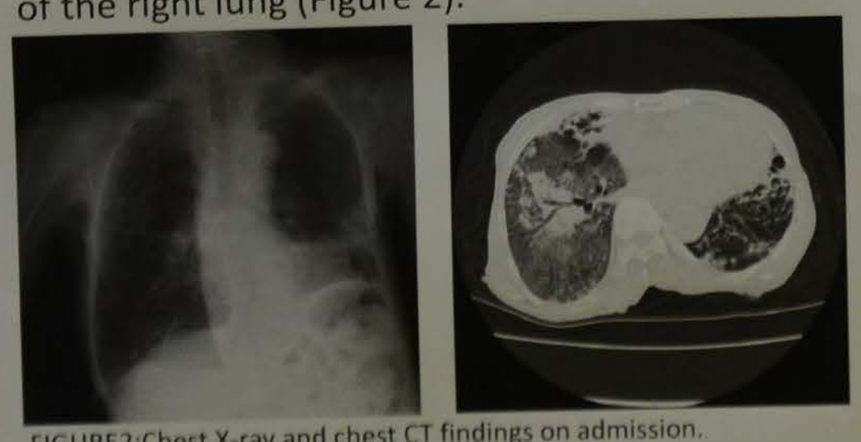


FIGURE2:Chest X-ray and chest CT findings on admission.

Her WBC count increased to $20 \times 10^3/mm^3$ with neutrophilic predominance (93.6%). Sputum Gram stain showed gram-positive branching rods suspected as a *Nocardia* species, and acid fast stain showed bacilli suspected as a *Mycobacterium* species. Both the *Nocardia* species and *M. intracellulare* were isolated from her sputum. Antimicrobial treatment was initiated with piperacillin/tazobactam and was changed to azithromycin on the 8th day of hospitalization, then to doripenem on the 15th day.



Colonies of *Nocardia cyriacigeorgica* in sheep blood agar(48h)

Her respiratory status improved, and antibiotics treatment was stopped on the 22nd day. The *Nocardia* species was identified as *N. cyriacigeorgica* by 16S ribosomal RNA gene sequencing. *N. cyriacigeorgica* was first classified in 2001 by Yassin et al. as a novel species [5]. Susceptibility testing of *N. cyriacigeorgica* was performed according to Clinical and Laboratory Standards Institute document M24-A, and it was regarded as sensitive to trimethoprim/sulfamethoxazole and imipenem (Table 1).

TABLE1:MIC of Nocardia cyriacigeorgica

Nocardia cyriacigeorgica	MIC (mg/ml)
Clarithromycine	>16 R
Imipenem	2 S
Levofloxacin	>4 R
Minocycline	4 R
Moxifloxacin	>2 R
Trimethoprim-sulfamethoxazole	≤0.5/9.5 S

S: sensitive; R: resistant

Conclusion: *Nocardia* species may cause co-infection with *M. intracellulare*, and we must be careful not to overlook this possibility. Therefore, performing sputum Gram stain is important. Furthermore, because the antimicrobial susceptibility pattern of *Nocardia* varies among species, correct identification of the species is important for the treatment of pulmonary nocardiosis.

Referenes

1. Kazuma Y *et al.* **Pulmonary nocardiosis caused by Nocardia cyriacigeorgica in patients with Mycobacterium avium cmplex lung disease: two case reports.** BMC Infect Dis 2014, 14:684-688
2. Daly AS *et al.* **Systemic nocardiosis following allogeneic bone marrow transplantation.** Transpl infect Dis 2003, 5:16-20.
3. Ohnishi H *et al.* **A case of pulmonary Nocardia farcinica infection in a patient with non-tuberculosis mycobacteriosis.** Kansenshogaku Zasshi 2000, 74:274-277.
4. Trinidad JM *et al.* **Coinfection by Nocardia asteroids and Mycobacterium avium- intracellulare in a patient with AIDS.** Enferm Infecc Microbiol Cln 1992, 10:630-631.
5.Yassin AF *et al.* **Nocardia cytiacigeorgica sp. Nov.** Int J Syst Evol Microbiol 2001, 51:1419-1423

Microbiology

PA-82

A case of *Yersinia psedotuberculosis* infection

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Introduction

Among infectious diseases related to *Yersinia*, *Yersinia pseudotuberculosis* (YPT) infection usually presents as a form of gastroenteritis though the various features, including Kawasaki disease-like symptom and acute renal failure, are known to manifest. Associations between exotoxin YPM (Y. pseudotuberculosis-derived mitogen) with such symptoms and superantigen activity have recently attracts attention.
We encountered a case of YPT infectious disease, described here.

[Patient] 14-year-old boy

[Complaint] fever and , abdominal pain

[Medical history/Family history] None

[Clinical history] He presented with fever in the morning and was diagnosed with upper respiratory tract inflammation at a nearby clinic. Cefdinil (CFDN) was prescribed. However, later that night, he was brought to our hospital emergency outpatient department because of a higher fever (40℃), chills, shivering, and vomiting, necessitating hospitalization. Because it was poor in ingestion. In addition, there was abdominal pain in the self-control. He was able to walk independently. He had not had any diarrhea or other bowel movement. He had eaten roasted meat 5 days before presentation. There was no epidemic disease in the neighborhood, he had not eaten raw food, he had not drunk well water, and he had not been involved in breeding of pets, including turtles.

[Hospitalization]

BT 40.6℃ SpO2 99%
Pulse 136 times/min BP 131/59mmHg
Awareness: Clear
Abdomen: Gurgle attenuation by a stethoscope
Flat and soft , no mass in palpation
No tenderness, No rebound tenderness

Laboratory data:

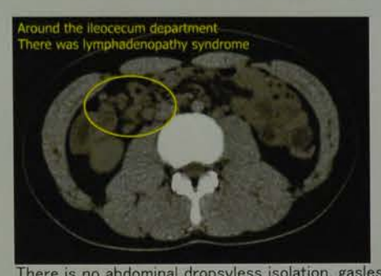
WBC	1100	/μL	ALP	698	U/L	Na	140	mEq/L
Hb	17.1	g/dL	AST	41	U/L	K	3.4	mEq/L
Ht	48.8	%	ALT	26	U/L	Cl	106	mEq/L
Plt	16.4×10⁴	/μL	LDH	207	U/L	Ca	9.4	mg/dL
CRP	4.25	mg/dL	γGTP	65	U/L	BUN	12.4	mg/dL
PCT	0.7	ng/mL	Amy	71	U/L	Cre	1.00	mg/dL
T-bil	1.3	mg/dL	CPK	117	IU/L	Glu	115	mg/dL
TP	7.2	g/dL						

Plain abdominal radiography



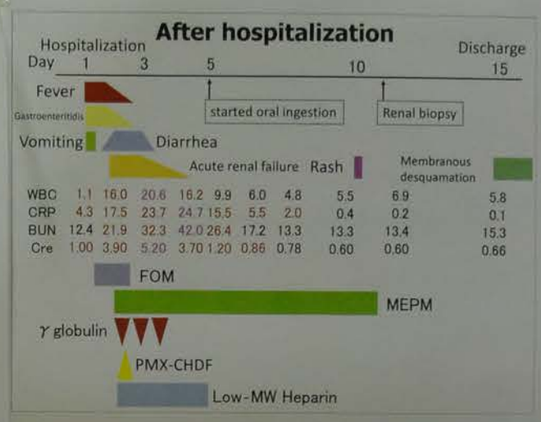
Gastrointestinal gas a bit
Surface formation was a frequent occurrence

Abdominal Computed Tomography



There is no abdominal dropsyless isolation, gasless abscess formation that there was extensive enteric fluid retention ,and slight congestion.

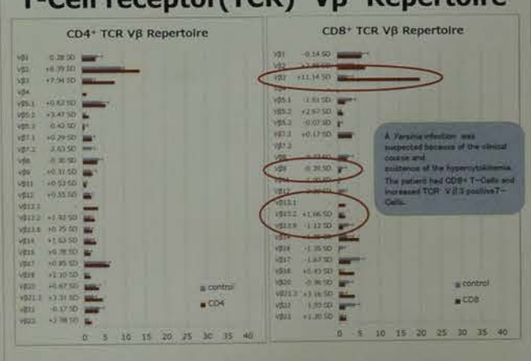
After hospitalization

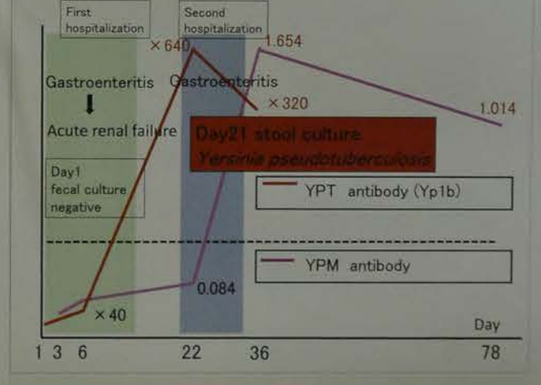


Cytokine	Standard Value	On admission	Before HD	Day3
Neopterin	<5.0nmol/L	14.5	≧111	64.0
IL-18	<500pg/mL	251	420	495
IL-6	<3.0pg/mL	19200	352	3
sTNF-R Ⅰ	484-1407pg/mL	13700	26200	4420
sTNF-R Ⅱ	829-2260pg/mL	29500	57000	21000

Urine biochemical test	Day1	Day2	Day3	Day4	Day6
FENa (%)	0.31	5.45	0.72	0.49	0.57
RFI(renal failure index)	0.42	7.67	1.00	0.68	0.80
NAG(IU/g･Cr)	18.9	10.38	6.11	4.84	6.48
α1MG(mg/g･Cr)	56.5	44.2	32.8	21.7	9.52
β2MG(μg/g･Cr)	570	80390	49544	8228	431

T-Cell receptor(TCR) Vβ Repertoire





Fecal culture (re-hospitalization)



CIN agar SS agar BTB agar (Lactose negative)

The culture was requested to confirm the absence of *Yersinia* spp. in Kanazawa Medical Center.
A small samples of stool was placed in 10mL of phosphate buffer solution and suspended with a spitz pipe. Several drops of the culture at 25℃ were suspended in CIN and SS agar. The suspension were stored 4 ℃ for 1 week, and then they were recultured in CIN agar and SS agar at 25℃
The patient was re-hospitalized., GNR "Oxydase negative" in CIN and SS agar ,thus *Yersinia* was suspected.

Differentiation *Yersinia* spp. and the bacteria were detected

	TSI (Sucrose)		VP 35℃	VP 25℃	Ure	Lys	Orn	motile 35℃	motile 25℃
Y. enterocolitica	A	A	(+)	−	+	+	−	+	−	+
Y. pseudotuberculosis	−	A	(−)	−	−	+	−	−	−	+



API20E code 1014112 (99.8%)
Yersinia pseudotuberculosis
and Identified as Serotype1

YPT infection

Diagnosis ①Bacteria were identified in the fecal sample (Low temperature culture (25℃、48hour))
②Increasing the serum antibody titer (single or paired serum)

YPM *Yersinia pseudotuberculosis*-derived mitogen (Superantigen)

<role> It is possible with the role that is important to the onset of systemic complications to bring about production sthenia of inflammatory cytokine and the T cell which has Vβ3, Vβ9, Vβ13 on the β chain of the antigen receptor

Renal pathology Tubulointerstitial renal disease

Yersinia infection

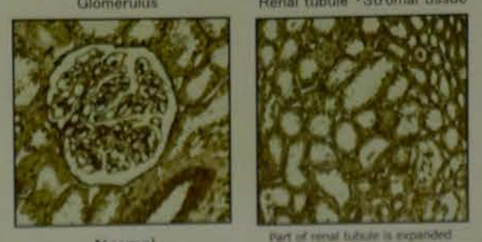
Yersinia enterocolitica and
Yersinia pseudotuberculosis (YPT)

Symptom Symptoms of gastroenteritis are importanat *Yersinia enterocolitica* But YPT does not generally have gastroenteritis symptoms and instead has various clinical manifestations and complications.

Complications	462 cases (Kawasaki Central Hospital)
Kawasaki disease	59 (12.8%)
Acute renal failure	51 (11.0%)
Erythema nodosum	35 (7.6%)
Ileocecum department lesion	13 (2.8%)

other : intussusception,juvenile rheumatoid arthritis, vascular purpura, acute renal failure, myocarditis, IgA nephropathy, HUS,TTP, uveitis

View from optical microscope (Day11)



Conclusion

① We encountered a case of gastroenteritis symptoms in a 14-year-old boy that suddenly progressed to renal insufficiency.

② The patient responded to treatment immediately, and his convalescence was good.

③ Disease-causing bacteria were not identified during the patient's first hospitalization, but the presence of *Yersinia* infectious disease was suspected based on the increase in cytokine blood symptoms and TCR Vβ3-positive T cells. In addition, the *Yersinia* infection was later certain, but the increase in YPM antibody was late than a conventional report in acknowledgment of an increase in serum antibody titers.

④ *Yersinia pseudotuberculosis* was identified from cultures obtained at the time of rehospitalization , but the source of infection could not be specified.

Acknowledgements

On the occasion of this study, I thank the people who had the cooperation such as detailed analyses heartily

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Microbiology

PA-83

Primary lymphocutaneous nocardiosis caused by minocycline non-susceptible *Nocardia brasiliensis* : a case report and analysis of antimicrobial susceptibility patterns of 10 clinical isolates.

PA-83

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Introduction

Nocardiosis is a rare infection and is clinically divided into cutaneous and systemic type; the former presents either as part of a disseminated infection or as a primary infection resulting from the invasion of a pathogen through an injury. It is well known that *Nocardia brasiliensis* is the most predominant causative pathogen in primary cutaneous nocardiosis. and It is also known that some *Nocardia* sp. has typical antimicrobial susceptibility patterns, therefore CLSI (M24-A2) recommend to use susceptibility patterns as a tool for predictive identification because of difficulty of correct identification of *Nocardia* sp. (Table 1)

Here, we report a case of primary lymphocutaneous nocardiosis due to atypical (minocycline non-susceptible) *N. brasiliensis* in an immunocompromised patient who was successfully treated with oral trimethoprim-sulfametoxazole (8 days) and minocycline (6 month). In addition, we also report antimicrobial susceptibility patterns analyzed 10 clinical *N. brasiliensis* isolates and discuss current status of susceptibility patterns.

Case report

Case

The patient was a 62-year-old man.
Chief complaint: Swelling and pain of the left upper elbow.
Past history: Hypopituitarism (treated with oral Ledercort ® at 4 mg/day) and diabetes.
History of present illness: In June 2015, he fell over in his garden and lacerated the left index finger.
The finger and the back of his left hand gradually became swollen. He visited a nearby dermatology clinic and received levofloxacin, but the swelling did not improve. He was referred to our hospital at 18 days after the injury (on June 30).

Findings on initial examination at our hospital

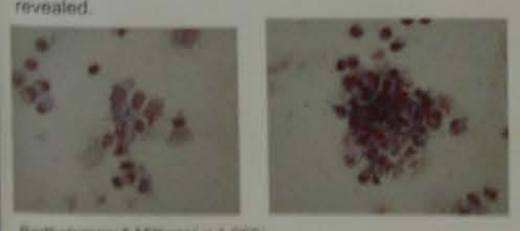


Blood analysis

WBC	9600 /μL	AST	18 U/L
RBC	495×10⁴ /μL	ALT	12 U/L
Hb	14.4 g/dL	LDH	320 U/L
PLT	22.1×10⁴ /μL	BUN	13 mg/dL
Neut	84.2 %	Cre	0.87 mg/dL
Lymp	10.8 %	TP	6.7 g/dL
Eosino	0 %	Alb	3.9 g/dL
Mono	4.6 %	CRP	2.3 mg/dL
		PCT	0.04 ng/dL
Glu	89 mg/dL	Na	143 mEq/L
Hb-A1c	6.0 %	K	4.0 mEq/L
β-D-glu	4 pg/mL	Cl	103 mEq/L

Bacteriologic examination①—A sample of pus was collected on June 30

Gram staining:
Nocardia-like Gram-positive actinomycetes were revealed.



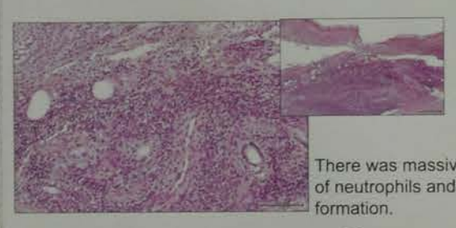
Barthylomew & Mittwer (× 1,000)

Culture:
After incubation for 48 hours in 5% sheep blood agar medium and chocolate agar medium, tiny white colonies with an earthy smell were appeared. From the nature of the colonies and the microscopy findings, *Nocardia* sp. was suspected.



Culture was done for 5 days in chocolate agar medium and blood agar medium under 5%CO2 at 35° C.

Histopathologic findings —skin biopsy specimen from the back of his left hand



There was massive infiltration of neutrophils and abscess formation.

Bacteriologic examination②

Antimicrobial susceptibility testing:
a) Method
Testing was performed by the CLSI broth microdilution method.
b) Results

CTRX	IPM	CPFX	MINO	LZD	ST	AMK	TOB	CAM
>2	>4	>2	4	2	≦10	≦4	≦1	>16
R	R	R	I	S	S	S	S	R

CTRX:ceftriaxon,IPM:imipenem,CPFX:ciprofloxacin,MINO:minocycline,LZD:linezolid,ST:sulfamethoxazole-trimethoprim,AMK:amikacin,TOB:tobramycin, CAM:clarithromycin,

N. otitidiscaviarum was suspected according to the "Expected antimicrobial susceptibility patterns" (Table 1) described in CLSI document M24-A2.
Table 1.

	CTRX	IPM	CPFX	MINO	LZD	ST	AMK	TOB	CAM
N. cyriacigeorgica	S	S	R	V	S	S	S	ND	R
N. abscessus	S	R	R	V	S	S	S	ND	R
N. nova complex	S	S	R	V	S	S	S	ND	S
N. farcinica	R	V	S	V	S	S	S	R	R
N. brasiliensis	S/R	R	R	S	S	S	S	S	R
N. otitidiscaviarum	R	R	S	V	S	S	S	ND	V

16S rRNA gene sequence analysis:
There was 99.93% homology (1457 bp) with *N.brasiliensis*, so the isolate was identified as *N. brasiliensis*.

Treatment

On July 1, after the findings of Gram staining were reported, oral administration of sulfamethoxazole-trimethoprim (Baktar combination tablets®:ST) was started at 4,800 mg/day. Redness and swelling improved. However, vomiting occurred and the medication was switched to minocycline (Minomycin ®:MINO) at 200 mg/day on July 9.After switching to MINO, there was further improvement and oral administration of MINO was continued for 6 months.



Two weeks after the start of the treatment

Six months after the start of the treatment

Additional test

Because the *N. brasiliensis* isolate detected in this case showed an atypical susceptibility pattern (MINO non-susceptible), the MIC and MBC were measured for 10 clinical isolates of *N. brasiliensis.*

Results

MIC

No.	CTRX	IPM	CPFX	MINO	LZD	ST	AMK	TOB	CAM
1	>256	>32	8	2	4	0.5	1	0.125	>64
2	16	>32	8	4	8	0.5	2	0.125	>64
3	>256	>32	8	4	4	0.5	2	0.25	>64
4	>256	>32	8	4	4	0.5	2	0.25	>64
5	>256	>32	8	4	8	0.5	2	0.5	>64
6	>256	>32	8	4	8	0.5	4	0.25	>64
7	8	>32	8	2	4	0.25	1	0.25	16
8	>256	32	8	4	8	0.5	4	0.25	>64
9	>256	>32	8	2	8	1	2	2	>64
10	32	2	4	2	4	1	2	0.25	>64

MBC

No.	CTRX	IPM	CPFX	MINO	LZD	ST	AMK	TOB	CAM
1	>256	>32	8	8	32	1	2	8	>64
2	16	>32	8	4	32	1	2	0.125	>64
3	>256	>32	16	4	>32	4	4	0.25	>64
4	>256	>32	8	8	16	0.5	2	0.25	>64
5	>256	>32	8	4	>32	0.5	4	0.5	>64
6	>256	>32	8	4	32	0.5	64	1	>64
7	8	>32	32	2	8	0.25	4	1	>64
8	>256	32	8	4	8	1	4	0.25	>64
9	>256	>32	8	2	8	8	2	2	>64
10	32	2	8	8	8	1	2	0.25	>64

MBC/MIC-Minocycline

No.	1	2	3	4	5	6	7	8	9	10
MIC/MBC	4	1	1	2	1	1	1	1	1	4

Discussion

1. Our patient

N. brasiliensis was initially suspected because he had a wound infection, but the isolate was MINO non-susceptible. Therefore, we thought that the infection might be due to *N. otitidiscaviarum.* MRI and radiological examination were performed to detect disseminated lesions, but there was no involvement of the central nervous system or the lungs. The isolate was identified as *N. brasiliensis* from its 16S rRNA gene sequence, so the final diagnosis was primary lymphocutaneous nocardiosis (local infection) caused by *N. brasiliensis*.

Despite the isolate being MINO non-susceptible, MINO was effective in our patient and the reasons for this outcome may be as follows: MINO has a bactericidal effect against *N. brasiliensis* (the MBC/MIC ratio was 1 in 7 out of 10 strains tested, while it was 2 in 1 strain and 4 in 2 strain), MINO achieves good tissue levels, and prior administration of ST for 8 days may have contributed to improvement.

2. Susceptibility profile of *N. brasiliensis*

When the susceptibility of 10 clinical isolates of *N. brasiliensis* was evaluated, all 10 isolates (100%) were found to be MINO non-susceptible (MIC ≥ 2 μg/ml).

Schlaberg et al. evaluated the susceptibility of 148 genetically identified *N. brasiliensis* isolates in the USA and reported that 76% were MINO non-susceptible, so the result of our present investigation is consistent with their findings. They pointed out that the susceptibility patterns of *N. brasiliensis* based on current taxonomy (genetic classification) were not consistent with the patterns based on conventional classification of bacterial species. Accordingly, re-appraisal of the susceptibility patterns based on current taxonomy is required.

Conclusion

Our experience in this case indicates that identification of *Nocardia* sp. can be incorrect if the bacterial species is determined according to M24-A2 susceptibility patterns, and we think that gene analysis is necessary for correct identification of bacterial species.

In addition, the M24-A2 susceptibility patterns of *N. brasiliensis* should be reconsidered based on the latest classification.

References: Robin Schlaberg, Mark A Fisher, Kimberley E Hanson. 2014. Susceptibility Profile of Nocardia Isolates Based on Current Taxonomy. Antimicrobial Agents and Chemotherapy. 58: 795-800

Disclosure of potential conflicts of interest: No potential conflicts of interest were disclosed.

Acknowledgments: This work was supported by grants from Charitable Trust Laboratory Medicine Research Foundation of Japan and Kurozumi Medical Foundation.

Microbiology

PA-84

A case of *Neisseria meningitidis* infection, isolated from blood cultures

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1)Hyogo College of Medicine Hospital, 2)Hyogo College of Medicine

【Introduction】

N. meningitidis is a Gram-negative diplococcus, causes the invasive meningococcal diseases. When entered into the bloodstream, it can spread to cerebrospinal fluid (meningococcemia) and induce meningitis. Without the proper treatment it may progress rapidly, resulting in death within a few hours. Reported here is a case of serious *N. meningitidis* infection that was isolated from blood cultures.

【Patient】

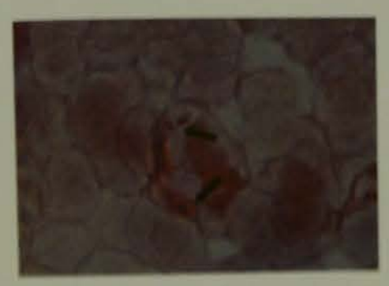
A 48-year-old male presented to another hospital with complaints of fever and respiratory discomfort. The patient developed to shock status with upper airway obstruction during the admission, thereby transported to our hospital. Laboratory results on admission are shown in 【Table 1】. With the suspicion of the septic shock, two sets of blood cultures were submitted.

【Table 1】 Laboratory results on admission

Biochemical			CBC			Coaglation test	
TP	(g/dL)	6.2	WBC	(/μL)	14,190	PT%	＜10
Alb	(g/dL)	3.4	RBC	(/μL)	553×10⁴	PT-Sec	61.4
CK	(IU/L)	1985	HGB	(g/dL)	18.3	PT-INR	5.77
Na	(mEq/L)	146	HCT	(%)	53.8	APTT (sec)	≧180
K	(mEq/L)	3.9	PLT	(/μL)	19,000	Fbg (mg/dL)	≦50
Cl	(mEq/L)	104	Seg	(%)	77.1	D-dimer(μg)	≧80
CRP	(mg/dL)	7.55	Lym	(%)	21.8	AT-Ⅲ (%)	44
Glucose	(mg/dL)	41	Mono	(%)	0.4		
			Eos	(%)	0.5		
Body tempature		38.9℃	Bas	(%)	0.2		

【Bacterial isolation】

Gram-negative diplococci were detected in blood cultures in 48 hours, and phagocytosis by neutrophils was observed on the staining.【Fig. 1】 On the next day, the bacteria formed colonies on blood agar【Fig. 2】, and identified as *N.meningitidis* by Microscan WalkAway (Beckman Coulter, Brea, CA). Susceptibility test was carried out using MIC2000 Frozen Plate (Eiken Chemical, Tokyo, Japan). The Results of susceptibility test are shown in【Table 2】.



【Fig. 1】
Gram-negative diplococci was eaten by neutrophil.



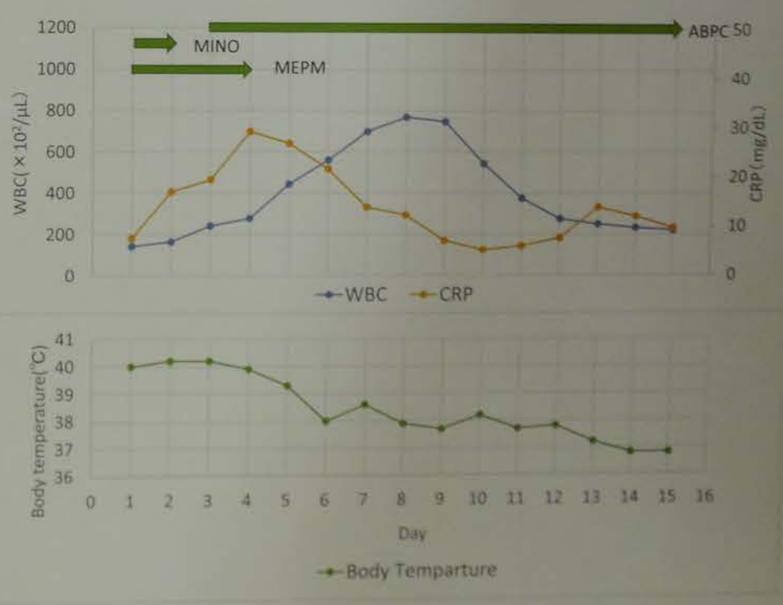
【Fig. 2】
Colonies on blood agar

【Table 2】 The results of susceptibility test

antimicrobial reagent	MIC	decision
PCG	≦0.06	S
ABPC	≦0.12	S
CTX	≦0.12	S
CTRX	≦0.12	S
CP	1	S
MEPM	≦0.06	S
AZM	≦0.25	S
MINO	≦0.25	S
CPFX	≦0.03	S
LVFX	≦0.03	S
ST	＞2	R

【Clinical Course】

He had been administered meropenem (2g × 2/day) for 3 days from admission, and then antimicrobial reagent was changed to ampicillin (2g × 4/day) according to the susceptibility test results. He had been administered it for 2 weeks, and recovered from meningitides. He was transferred to another hospital.



【Fig. 3】 Clinical course

【Discussion】

Immediate diagnosis and early treatment is important for the bacterial infections, especially for the meningococcal infection. In the case presented, we had received the information of the suspicious meningococcal infection before the culture started, which resulted in the quick identification of the bacterial strain and led to the appropriate selection of the anti-biotic reagent.

【Conclusion】

This case reconfirmed the importance of cooperation of the medical laboratory with the clinical sections.

We have no financial relationships to disclose.

Microbiology

PA-85

A case of granulomatous mastitis caused by Corynebacterium tuberculostearicum infection in a nulliparous young woman

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1) Dept. of Central Laboratory Medicine, Nara Prefecture General Medical Center, Nara, Japan
2) Department of Laboratory Medicine, Tenri Hospital, Nara, Japan

Introduction

Recently, infection with Lipophilic *Corynebacterium* such as *C. kroppenstedtii* and *C.tuberculostearicum* has attracted attention. We have experienced a case that was isolated from *C. tuberculostearicum* which was the same as lipophilic *Corynebacterium* from a case of mastitis.

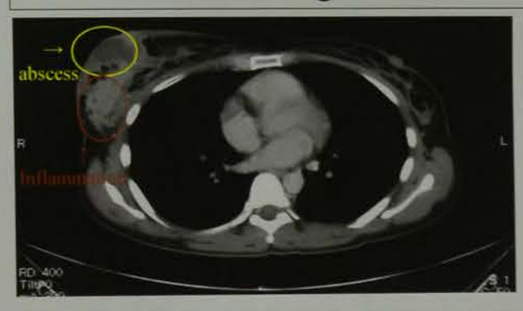
Case

A 34 year old woman had had swelling and pain in her right mammary gland for one month. As a result of various inspections, the breast cancer was negative and the administration of antibiotics was started. However, her condition turned worse and the fever occured, therefore, she was hospitalized. As a result of mammary gland puncture, lipophilic *Corynebacterium* was detected from aspirated pus by the bacteriological examination. Based on these, drainage was performed and levofloxacin was prescribed, so that the symptoms of this patient were improved.

Biochemical data

WBC	10.0	×10^3/μl	CRP	9.58	mg/dl	LDH	133	IU/l
Neu	75.7	%	Na	136	mEq/l	CK	45	IU/l
Lym	16.0	%	K	3.9	mEq/l	BUN	9.9	mg/dl
Mon	4.9	%	Cl	100	mEq/l	CRE	0.66	mg/dl
RBC	3.44	×10^6/μl	TP	6.5	g/dl	T-BIL	0.74	mg/dl
Hb	10.4	g/dl	Alb	3.4	g/dl	γGTP	20	IU/l
Hct	31.9	%	AST	12	IU/l	ALP	171	IU/l
Plt	39.8	×10^4/μl	ALT	13	IU/l	Amy	57	IU/l

Radiological examination



Computed tomography shows swelling of the right mammary gland, fluid collection and necrotic change between the pectral muscle and subcutaneous, and abscess formation was suspected.

Microbiological examination

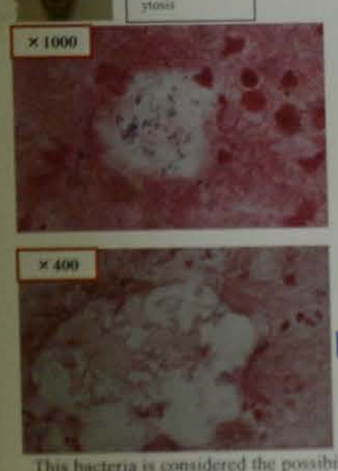
Gram stain



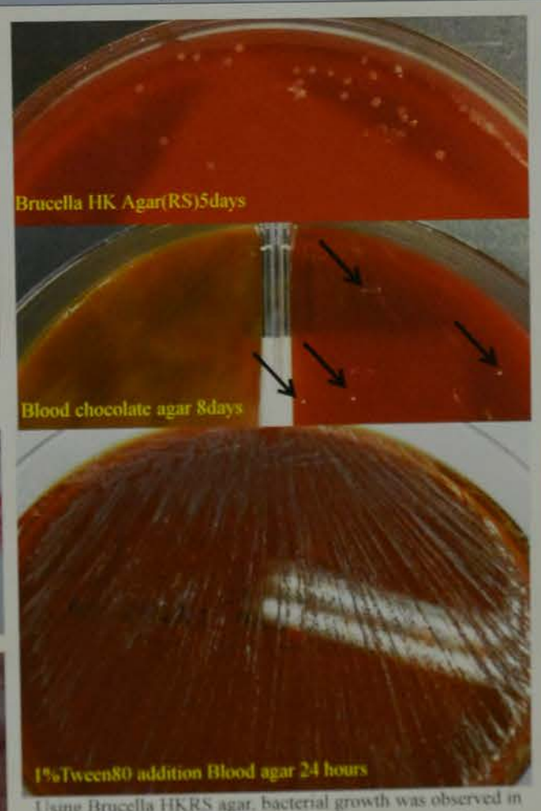
	Color / Dark brown
Smell	−
GPC	−
GNC	−
GPR	+
GNR	−
WBC	4 +
RBC	4 +
Epi	−
Phagocytosis	−



Bacteri was confirmed that are concentrated in the lipid erea.



This bacteria is considered the possibility of C.kroppenstedtii from the infected area and the Gram stain findings, we had carried out for long time culture.



Using Brucella HKRS agar, bacterial growth was observed in five days, while only micro-colonies were seen in eight days by Blood chocolate agar. Obvious growth enhancement was observed when 1% Tween80 added blood agar was used, confirming the existence of Lipophilic Corynebacterium.

Biochemical identification

VITEK2 ANC (SYSMEX bioMérieux Co.,Ltd.)
No : 6363100400405
C. amycolatum *C. minutissimum* *C. striatum*

Api Coryne (SYSMEX bioMérieux Co.,Ltd.)
No : 2100105
C. striatum *C. amycolatum*

C. kroppenstedtii are not included in the database of VITEK2 and Api Coryne

From the above, we thought that there is a high possibility of *C. kroppenstedtii*.

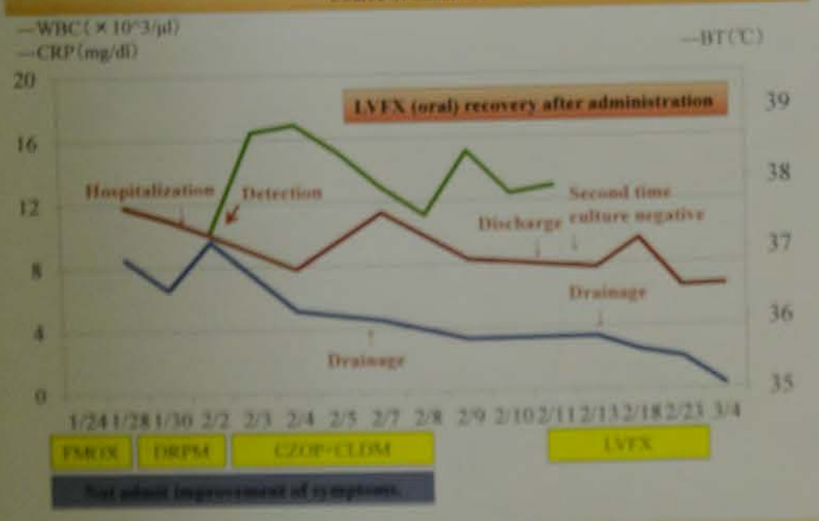
Chemical sensitivity test

EIKEN CHEMICAL CO.,LTD. DP32

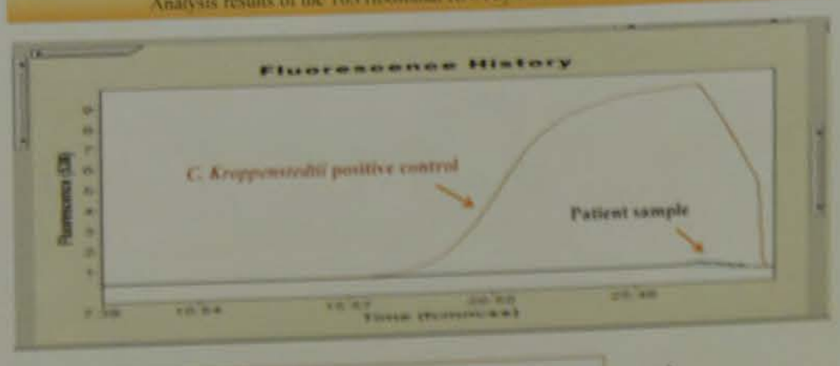
PCG	0.12	S	*No effective*
CTRX	1	S	
CFPM	0.25	S	
IPM/CS	<=0.25	S	
GM	<=0.25	S	Judgment based on the CLSI M45-A2
EM	>4	R	
CLDM	>2	R	
MINO	<=2	S	*Effective*
LVFX	<=0.25	S	
VCM	<=0.5	S	

Chemical sensitivity test shows that a large part of the drugs are effective against Lipophilic Corynebacterium. But mastitis caused by Lipophilic Corynebacterium is frequently refractory to β-lactam antibacterial agents by its water-soluble nature. Therefore, we recommend the use of minocycline and levofloxacin.

Course of treatment



Analysis results of the 16S ribosomal RNA by Real-time PCR method



【Primer】
Corynebacterium kroppenstedtii 16S rRNA gene (2760bp)
Forward primer 5'-CCATCTCTGTAGGTGGTGTGG-3'
Reverse primer 5'-GAAGGCTTCGTCATGGCTG-3'

→ Negative

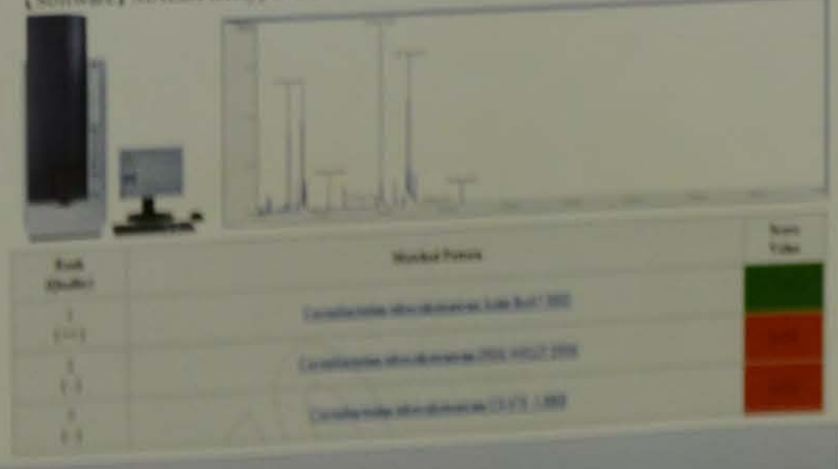
Analysis by Broad-range PCR of 16S Ribosomal RNA and sequence



Corynebacterium tuberculostearicum
16S ribosomal RNA
Identities = 1511/1512 (99.9%)

Analysis by mass spectrometry

【Analyze device】MALDI Biotyper (Bruker Daltonics K.K.)
【Software】MALDI Biotyper 3.0



Rank (Scaffold)	Matched Pattern	Score Value
1 (1+1)	*Corynebacterium tuberculostearicum* Acute Bed C 3005	
2 (1+1)	*Corynebacterium* sp. DNF 00727 2956	
3 (1+1)	*Corynebacterium* CLCL 1.3005	

Discussion

C. tuberculostearicum is likely to be a pathogenic bacterium in this case. Because *C. tuberculostearicum* is not included in the database of ApiCoryne and VITEK2, these can not identify the species accurately. In addition, it was reported that ApiCoryne (bionumber: 2040104) identified *C. kroppenstedtii* incorrectly as *C. argentoratense*. We consider that PCR and mass spectrometry are necessary in order to distinguish the two species accurately. Mastitis caused by Lipophilic Corynebacterium is frequently refractory to β-lactam antibacterial agents by its water-soluble nature. Therefore, reporting as Lipophilic Corynebacterium is a high clinical significance, although the species is not identified.

Nara Prefecture General Medical Center

Microbiology

PA-86

Two cases of *Clostridium tertium* infection and identified by MALDI-TOF mass and 16S rRNA sequencing analysis

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1) Section of Infection Diseases, Laboratory Division of Clinical Support, Hiroshima University Hospital
2) Project Research Center for Nosocomial Infectious Diseases, Hiroshima University
3) Research Facility, Hiroshima University Faculty of Dentistry
4) Division of Laboratory Medicine, Hiroshima University Hospital
5) Department of Infectious Diseases, Hiroshima University Hospital

Introduction

Clostridium tertium is an endospore forming anaerobic Gram-positive bacilli found in the soil and the gastrointestinal tract of humans. The organism is able to grow at aerobic condition. It is easily decolorized in Gram-stained smears, which often leads to mistaken identification as a Gram-negative organism [1]. We report here two cases of *C. tertium* isolated from blood culture, one of which was successfully identified by using matrix-assisted laser desorption-ionization time-of-flight mass spectrometory (MALDI-TOF MS) analysis and subsequent 16S ribosomal DNA sequencing.

Discussion

C. tertium was considered a pathogenic bacterium in the case because it had been isolated from blood culture and because of the neutropenia. *C. tertium* is resistant to the third and fourth generation cephalosporins and aminoglycosides, and vancomycin (VCM) and/or imipenem are often used therapeutically to treat *C. tertium* infection [2),3)]. Meropenem and VCM were administered for seven days and the patient survived. Given the difficulties in differentiating between the several *Clostridium* species, our work underscores the necessity of using a variety of techniques to assist clinical identification.

Conclusion

Our study suggests identification of bacterial species by combining MALDI-TOF MS with 16S rRNA gene sequencing provides a reliable means to obtain rapid identification of pathogens in the clinical setting, removing the ambiguity around poorly differentiable strains such as those of *Clostridium* species.

Case 1

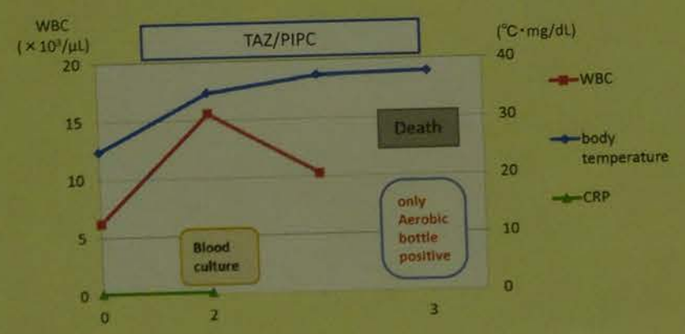
A 50s man

【Chief complaint】low temperature, hypothermia and state of shock

【Past medical history】diabetes, liver cirrhosis, alcohol dependence

【Findings on admission】body temperature: 24.8℃, blood pressure: 177/59 mmHg, pulse 48/min, respiration rate: 10/min

【Course】 Day 2 after hospitalization, 2 sets of blood culture were drawn, and the antimicrobial treatment started with tazobactam/piperacillin (TAZ/PIPC). Day 3, he died.



【Identification course】

Blood culture only Aerobic bottle positive → ①Gram stain(blood) Gram-negative bacilli **Report: Gram-negative bacilli** → ②Culture Colony grew on blood agar plates, not on BTB agar plates. **Add the anaerobic culture** → Colony grew in both aerobic and anaerobic culture

③Gram stain(colony) Spore-forming Gram variable bacilli → Suspected *Clostridium* spp. Identified by VITEK2 Compact and RapID ANA Ⅱ → ④Result *C. tertium* → ⑤ MALDI TOF MS analysis *C. tertium*

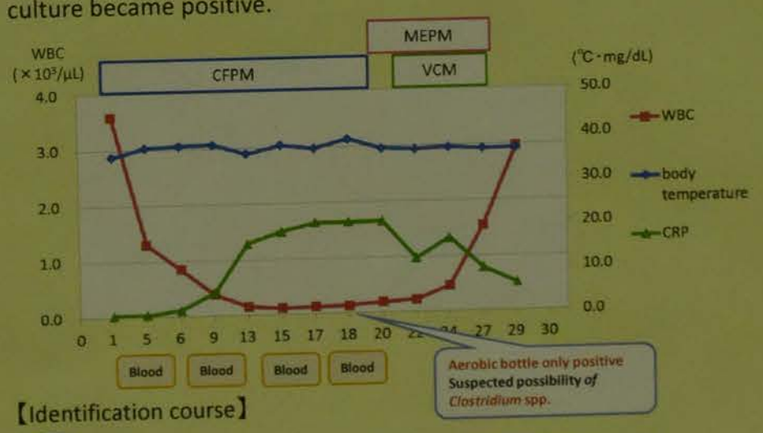
Case 2

A 60s man

【Chief complaint】fever 【Past medical history】diabetes, hepatitis B

【History of present illness】Three or four months before, he had been diagnosed acute myelogenous leukemia, and hospitalized for remission induction therapy.

【Course】Two days after the start of chemotherapy, he started fever up and his leukocyte count declined, and antimicrobial treatment started with cefepime (CFPM) but resulted in no effect. The 20 day of illness, blood culture became positive.



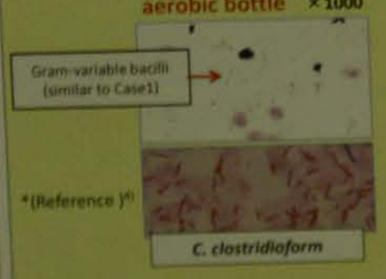
【Identification course】

Blood culture only Aerobic bottle positive → ①Gram stain(blood) Gram-variable bacilli similar to Case1 **Report: Possibility of *Clostridium* spp.** → ②Culture Suspected *Clostridium* spp. **sub-cultured in aerobic and anaerobic** → Colony grew in both aerobic and anaerobic culture

③Gram stain(colony) Spore-forming Gram variable bacilli → Suspected *Clostridium* spp. identified by VITEK2 Compact and RapID ANA Ⅱ → ④Result *C. clostridioform* → ⑤ MALDI TOF MS analysis 16s rRNA gene sequencing analysis *C. tertium*

【Blood culture Gram stain】(Case 2)

aerobic bottle ×1000



① Gram-variable bacilli similar to case1
⇒**possibility of *Clostridium* spp.**

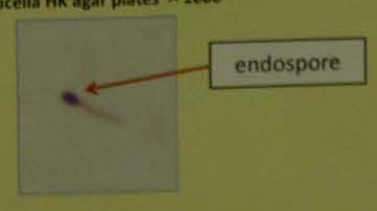
【colony】(Case 2)



② **sub-cultured in aerobic and anaerobic**
colony grew in both aerobic and anaerobic culture (case1 similary)
⇒**colony gram stain**

【colony Gram stain】(Case 2)

Brucella HK agar plates ×1000



③ endospores were observed, suspected
anaerobic Gram-positive bacilli (*Clostridium* spp.)

【Bacteriological examination】

Bacteriological examination	Case 1	Case 2
catalase test	negative	negative
gas produce	positive	positive
automated identification apparatus VITEK2 Compact	*C. tertium*	*C. clostridioform*
identification kit Rap ID ANA Ⅱ	*C. tertium*	*C. clostridioform*

④Result of VITEK2, Rap ID was different from Gram-stain image
⇒**genetic test**

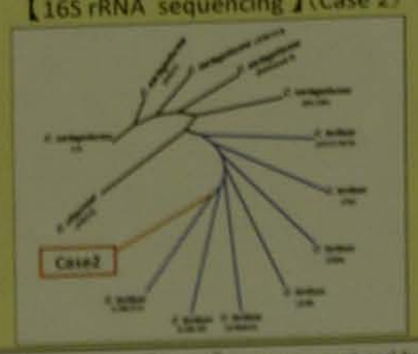
not form endospores, observed as the football-form (*)

【MALDI-TOF MS, 16S rRNA sequencing analysis】

	Case 1	Case 2
MALDI Biotyper analysis	*C. tertium* (Score Value 2.03)	*C. tertium* (Score Value 2.093)
16S rRNA gene sequencing analysis		*C. tertium* Homology 100% match

Meaning of Score Value
2.000 – 2.299 : secure genus identification, probable species identification

【16S rRNA sequencing】(Case 2)



⑤16S rRNA sequence of "case 2" strain was analyzed by BLAST search, and phylogenetic tree was created with strains showing more than 98% homology

【Antibiotic susceptibility testing result】

Case 1

drug	MIC (μg/ml)	decision
ABPC	1	I
PIPC	16	S
SBT/PIPC	8	S
CTX	16	S
CZOP	16	S
CMZ	1	S
IPM	1	S

Resistant to third and fourth generation cephalosporins

Case 2

drug	MIC (μg/ml)	decision
ABPC	1	I
PIPC	1	I
SBT/PIPC	8	S
CTX	>32	R
CTRX	>32	R
CZOP	16	S
CMZ	<=1	S
IPM	<=0.25	S
MEPM	<=0.25	S
VCM	<=1	

Reference

1) D.L. Miller, *et al.* Clin Infect Dis 2001;32:975-8.
2) Steyaert S, *et al.* Clin Microbiol 1999;37:3778-9.
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Microbiology

PA-87

A case of successful isolation of *Clostridium tetani* from drainage

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2) Department of Medical Technology and Sciences, International University of Health and Welfare, Fukuoka, Japan.

Case presentation

【Back ground】
- An 83-year-old woman
- Chief complaint: abdominal distention and melena
- Medical history: Fibroid, Cerebral infarction, Asthma

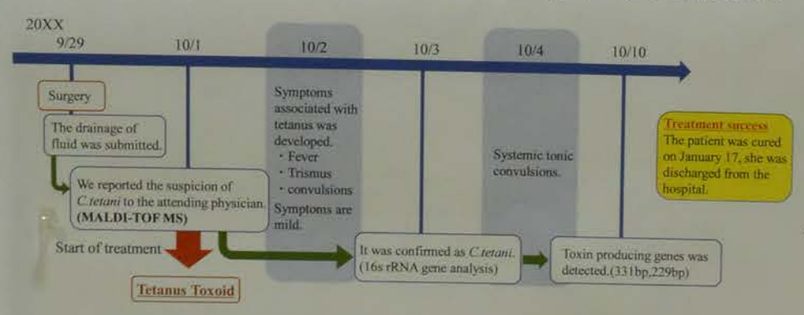
【History of present illness】
- Found bleeding from abdominal distension and genitals on September 28, she was diagnosed with inflammatory bleeding from the ovary on a transvaginal echo at the gynecology.
- The same day, she was emergency admission to our hospital due to worsening of symptoms.

Characteristic of *C. tetani*

- It is an obligate anaerobic gram-positive bacillus, distributed in soil around the world.
- It is very rare to be isolated and identified in the microbiology laboratory.
- Gram staining and spore staining of colonies revealed distinctive drumstick appearance.
- It forms swarming colonies on blood agar medium.
- It produces tetanospasmin as a neurotoxin and causes specific clinical symptoms such as trismus and systemic tonic convulsions.
- It is a pathogenic bacteria of tetanus, and early medical examination and treatment is necessary because of severe symptoms and a high mortality rate.

Overview

9/29: CT showed intestinal gas and intestinal fluid retention. She was diagnosed with ileus.
9/30: Sepsis and DIC was recognized by the blood drawing, and emergency surgery was performed.



Conclusion

- We experienced a case of the isolation of the Clostridium tetani from the post-operative drainage.
- MALDI-TOF MS is very useful, because it can identify very small amounts of bacteria.
- It is difficult to identify the allied genotype bacteria by MALDI-TOF MS, and the 16S rRNA gene analysis needs to be carried out in parallel.
- We reported to the attending physician quickly, resulted in contributing to the early diagnosis and treatment of tetanus.

Identification by MALDI-TOF MS

A few amounts of colonies in "KBC" anaerobic rabbit blood agar treated with ethanol-formic acid extraction method and identified by a MALDI-TOF MS.

Rank (Quality)	Matched Pattern	Score Value	NCBI Identification
1 (★★)	Clostridium tetani type 1 1049 NCTC 2797 BOG	2.006	tetani
2 (★★)	Clostridium tetani DSM 11744 VML	2.007	tetani
3 (★)	Clostridium tetani 1089 ATCC 10779 BOG	1.812	tetani
4 (★)	Clostridium tetani DSM 11745 VML	1.715	tetani
5 (-)	Streptococcus pneumoniae ATCC 49619 THL	1.582	pneumoniae
6 (-)	Saccharothrix mutabilis ssp capreolus HKI 264 HKJ	1.555	mutabilis
7 (-)	Acidothermus acidophilum B345 UTL	1.537	acidophilum
8 (-)	Lactobacillus harbinensis DSM 16991T DSM	1.529	harbinensis
9 (-)	Saccharothrix mutabilis ssp mutabilis HKI 371 HKJ	1.527	mutabilis
10 (-)	Paenibacillus illinoisensis DSM 11734 DSM	1.525	illinoisensis

It reported *the Clostridium tetani* immediately to the attending physician.

	Identification	Amount of bacteria
1	*Clostridium tetani*	very few
2	*Klebsiella pneumoniae*	1+
3	*Lactococcus garvieae*	a few
4	*Clostridium sporogenes*	1+

16s rRNA gene analysis method

DNA extraction (Cica Geneus DNA Extraction Reagent) → PCR → Electrophoresis → Purification of PCR products (Cica Geneus PCR Prep Kit) → Cycle Sequencing → Purification of Cycle Sequencing products (BigDye Xterminator Purification Kit) → Direct sequencing (Genetic Analyzer3130)

Description	Max score	Total score	Query cover	E value	Ident
Clostridium tetani E88 strain Massachusetts 16S ribosomal RNA, complete sequence	2669	2669	99%	0.0	99%
Clostridium tetani strain NCTC 279 16S ribosomal RNA gene, partial sequence	2584	2584	99%	0.0	98%
Clostridium cochlearium strain JCM 1396 16S ribosomal RNA gene, partial sequence	2580	2580	99%	0.0	98%
Clostridium cochlearium strain ATCC 17787 16S ribosomal RNA gene, complete sequence	2468	2468	98%	0.0	97%

Detection method of tetanospasmin gene



M: size marker, 100bp ladder
Lane1: tetanospasmin gene amplified by the two primers GAT1 and GAT2
Lane3: tetanospasmin gene amplified by the two primers GAT5 and GAT6
Lanes2,4: Negative control

It was carried out to search for the toxin-producing gene by PCR with the use of Primer pairs.
※Primer pairs (GAT1 and GAT2, GAT5 and GAT6)

↓

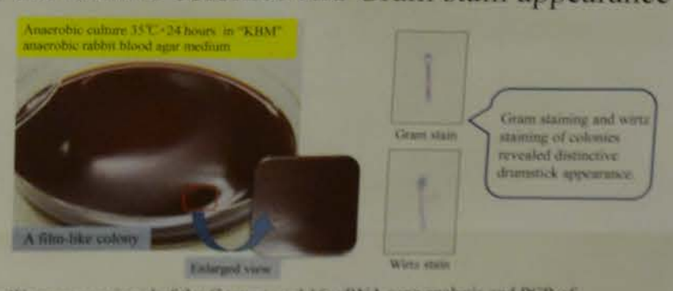
It showed a band in the vicinity of the 331bp and 229bp

↓

It was identified as Tetanospasmin

GAT1: 5'-gatgatacgtatgccaataacc-3'
GAT2: 5'-tttaggcctcaoctgctacattg-3'
GAT5: 5'-ctacatggtttatacggaatgcagg-3'
GAT6: 5'-gaicattgcagctagtgacttgac-3'

Pure culture colonies and Gram stain appearance



・We were convinced of the *C.tetani*, and 16s rRNA gene analysis and PCR of toxin-producing genes was performed.

Laboratory Data on Admission

Hematology		Hemostasis		Biochemistry				Blood Gas Analysis	
WBC	9370/μl	PT(sec)	13.4sec ↑	ALB	3.7g/dl	AMY	64U/l	pH	7.362
NEU%	91.7%	PT(%)	74.6%	T-Bil	2.4mg/dl ↑	P-AMY	52U/l	pCO2	27.5mmHg ↓
LY%	3.9%	PT(INR)	1.17	AST	35U/l ↑	UA	8.8mg/dl ↑	pO2	75mmHg
MO%	4.4%	APTT	29.9sec	ALT	16U/l	BUN	5.0mg/dl ↓	stHb	14.3g/dl
RBC	438×10⁴/μl	D-D dimer	1.9μg ↑	ALP	168U/l	CRE	1.17mg/dl ↑	Hct	43.8%
Hgb	14.1g/dl			LDH	388U/l ↑	Na	139mEq/l ↓	cNa+	138mEq/l ↓
Hct	39.1%			γ-GT	18U/l	K	3.2mEq/l ↓	cK+	3.1mEq/l ↓
MCV	89.1fl			CPK	663U/l ↑	Cl	91mEq/l ↓	cCl-	92mEq/l ↓
MCH	32.2pg			CRP	10.70mg/dl ↑			cCa2+	2.13mEq/l ↓
MCHC	36.1%								
PLT	17.6×10⁴/μl								

Gram stained image and Culture method of drain fluid



Microbiology

PA-88

Introduction

Discussion

Conclusion

Case: A 23s man

Microbiological examination
[ODH and MALDI-TOF MS-Biotyper analyses]

[Antibiotic susceptibility testing result]

Microbiology

PA-88

Mycobacterium abscesssus in blood culture of a patient with a long-term indwelling CVC (case report)

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3) Project Research Center for Nosocomial Infectious Diseases, Hiroshima University
4) Research Facility, Hiroshima University Faculty of Dentistry
5) Department of Infectious Diseases, Hiroshima University Hospital

Introduction

M. abscessus is a rapidly growing mycobacterium (RGM) present in soil and water and causes skin and lung in infections in immunocompromised patients[1]. We report a case that needed time to make a diagnosis of *M. abscessus* infection. The organism was not detected from the culture of the catheter insertion site, but from the blood culture and *M. abscessus* was identified later.

Discussion

When blood culture becomes positive, it is important to consider a possibility of acid fast bacterial infection and perform acid fast staining and culture. DDH Mycobacteria suggested the isolate *M. chelonae* but MALDI-TOF MS-Biotyper analysis, sequencing analyses of 16S rRNA and *hsp*65 suggested *M. abscessus*. DDH Mycobacteria have been reported to occasionally misdetect[2], and we thought it was necessary to use other methods.
MIC was measured by microdilution method or E-test. Antibiotic susceptibility was defined according to the criteria of CLSI (M24-A2). MIC values of E-test is reported to be higher than those of microdilution method[3]. In this case, striking differences were observed in MIC values of MINO and AMK. *M. abscessus* is resistant to anti-tubercular agent. ATS/IDSA performs antibiotic susceptibility testing of the CAM, IPM, CFX, aminoglycoside antibiotics, and new quinolone and recommends to perform multidrug therapy based on results[4]. Of note due to lack of oral medicine for the treatment, physicians often encounter difficulty in the treatment of M. abscessus infection. Generally resection of the localized lesion and combination of the multidrug therapy show good prognosis.

Conclusion

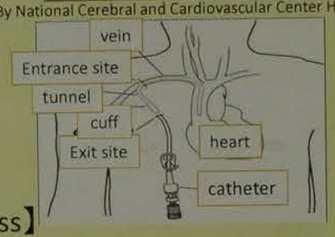
In this case, MALDI-TOF MS was useful for the identification of an acid fast bacteria isolated from blood culture, and the doctor was able to choose an appropriate antimicrobial agent without delay according to the bacterial information we submitted to the clinical side.

Case : A 23s man

《Chief Complaint》 fever, malaise
《Present illness》 He was visiting University hospital at cardiovascular internal medicine for treatment of primary pulmonary arterial hypertension.
Hickman catheter was detained approximately two years ago and started Floran constant injection.
《Physical findings》 Body temperature: 39℃,
Blood pressure :97/58mmHg
SpO_2 98%（home oxygen therapy）
《Social history》 No smoking, alcohol :occasionally



By National Cerebral and Cardiovascular Center HP



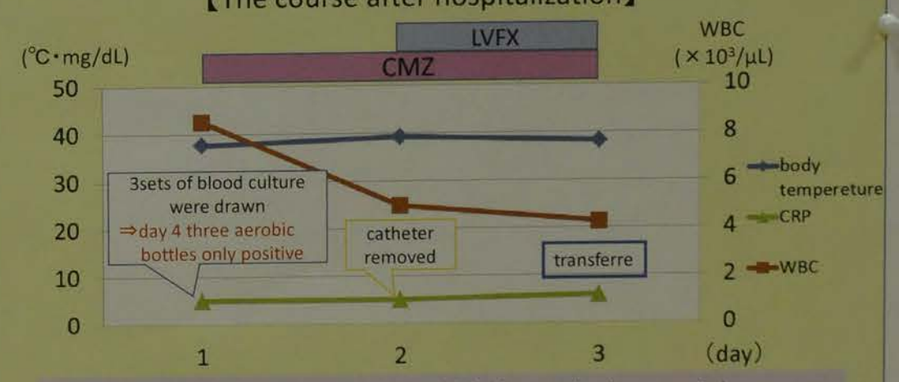
【The course before hospitalization】

	Symptom	General bacterial culture	antimicrobial agent
10 months ago	He detected a feeling of itch and an exudate in catheter exit site	Decoction (negative)	CCL
2 months ago	Sharp pain and bleeding →admission He left the hospital after improvement of the symptom	Decoction (negative)	CMZ LVFX
2 days ago	Diabetes and high blood pressure with 38℃		

【Identification process】

day 1	Three aerobic bottles positive
	Gram-staining of the blood culture（Figure 1）negative？
day 3	Culture（Figure2） Sub-culture on blood and chocolate agar plates yielded shiny white colonies
	Gram-staining of the colony demonstrated Gram variable bacilli
	Acid-fast staining of the colony demonstrated acid fast bacilli
	We suspected acid fast bacteria as etiological agent →species identification and antimicrobial susceptibility
day 4	DDH Mycobacterium suggested *M. chelonae*（Figure 3）
2 months later	Sequencing analysis and MALDI-TOF MS suggested *M. abscessus*

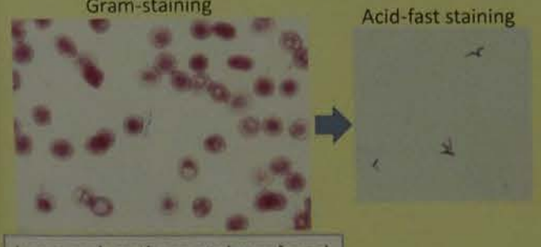
【The course after hospitalization】



※Surgical procedures such as washing and debridement has been carried out after changing hospital

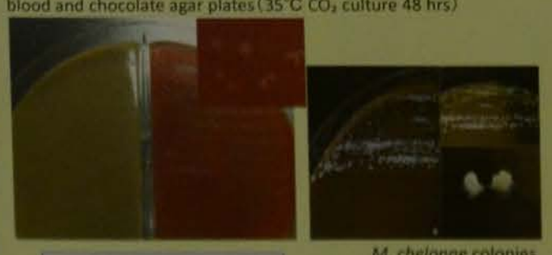
Microbiological examination

【 Blood culture Gram stain 】(Figure 1)
Aerobic bottles ×1000



Lump such as the crystal was found

【Culture photograph】(Figure 2)
blood and chocolate agar plates（35℃ CO_2 culture 48 hrs）



Small white colonies growth　　*M. chelonae* colonies (reference)[5]

【DDH and MALDI-TOF MS-Biotyper analyses 】(Figure 3)

	case
DDH methods	*M. chelonae*
MALDI Biotyper (Biflex Ⅳ)	*M. abscessus* (Score Value 2.348)



Absorbance
M. chelonae 0.101
M. abscessus 0.052

Relative similarity was 31% (less than 70%)
M. chelonae

Discrepancy between DDH and MALDI-TOF MS exists

【Antibiotic susceptibility testing result 】

Drug	MIC(μg/mL)	E-test MIC(μg/mL)
CAM	<=1	0.5
AMK	2	8
ST	>40	>32
CPFX	>2	>32
IPM	>4	>32
CFX	>8	
MINO	<=2	>256

CLSI（M24－A2）
resistance
sensitivity
No standard

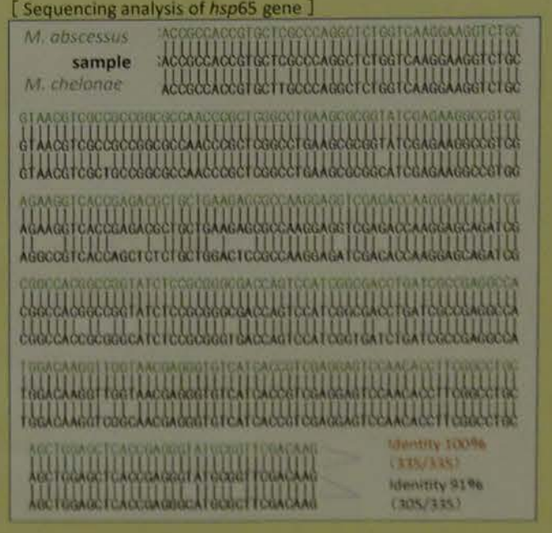
【 Sequencing analysis 】(Figure 4)

Sequencing analysis of 16S rRNA	*M. abscessus* *M. chelonae*

↓ Because homogeny was high, identification was impossible

Sequencing analysis of *hsp65*	*M. abscessus*

[Sequencing analysis of *hsp*65 gene]



Reference

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Microbiology

PA-89

A case of chromoblastomycosis
we could identify based on results of fungal culture

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A case of chromoblastomycosis we could identify based on results of fungal culture

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Department of Clinical Laboratory, Kanazawa University Hospital

Introduction

Chromoblastmycosis is one of the deep dermatomycosis that is representative in Japan and is caused by dematiaceous fungi. Soil-dwelling fungi invade from a wound of the skin and develop it. Dematiaceous fungi produce verrucous and plaque appearance, and histopathologically sclerotic cells are classified as chromoblastmycosis.

We report a case of chromoblastmycosis that we identify the fungus as *Phialophora verrucosa* (*P.verrucosa*) by results of fungal culture.

Case report

The patient was a 77-year-old man and a farmer. He had not been under an immunocompromised condition. He had noticed a small nodule on the back of the right hand 1 year before he visited another hospital. However diagnosis was difficult. Then he was referred to Kanazawa University Hospital 2 months later and the culture examination and the skin biopsy were performed.

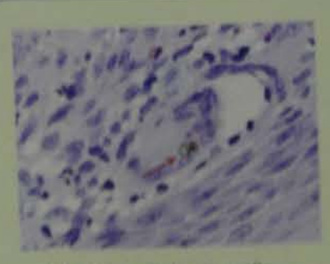
Clinical findings



The nodule was a crusted erythematous plaque, 35 × 50 mm in size on the back of the right hand .

Pathological findings

Histopathological examination of the biopsied skin showed infiltration of neutrophils in the dermis and infiltration of lymphocytes, neutrophils, plasma cells, eosinophils, and multinucleated giant cells in the subepithelium. Further, there were sclerotic cells with a blackish brown thick cell wall among the multinucleated giant cells. By finding sclerotic cells, this case was diagnosed of chromablastomycosis.



HE stain : Sclerotic cells with a blackish brown thick cell wall among the multinuecleated giant cells.



Grocotto stain : Sclerotic cells which was connected in the shape of beads.

Culture results

Standard bacteria（35 ℃, 48 houres (CO_2)） : negative
Acid-fast bacteria（37 ℃ &RT, 8 weeks） : negative
Fungus （30 ℃, 2 weeks） : On the 10th day, black cluster growed on Sabouraud dextrose agar. Next we subcultured it on potato dextrose agar, then we accompanied with a slide-culture.

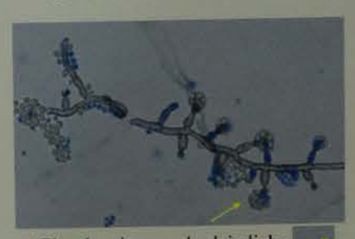


On potato dextrose

Colony findings

- 30 ℃ for 21 days
- Size in 15 mm, blackish brown
- Grayish aerial hyphae grow thickly on the surface

Slide-culture （Lactophenol-cottonblue stain）



Flask-shaped phialide



Cup-shaped collarette

We identified the fungus as *Phialophora verrucosa* by these results.

Treatment

The patient was treated with oral itraconazole (100mg/day, for 5 months) without debridement and the lesion healed. He is now an outpatient without recurrence after the treatment.

Conclusion

We reported a case of comparatively rare kind of chromoblastmycosis in Japan on the back of the right hand lesion. Histopathologically sclerotic cells were classified as chromoblastmycosis. We were able to identify it with comparatively rare *P. verrucosa* by a colony property for culture, optical microscope views .

Later, it was identified as the same fungus by gene analysis at Chiba University Medical Mycology Reserch Center.

Blast analysis

The base sequence of the ITS region was decided. As a result of blast analysis, it shared homology more than 98% with 11 base sequence that is registered as *P. verrucosa* in a database. Therefore, this strain was identified as *P. verrucosa* in PCR.

TTTCCGTAGGTGAACCTGCGGAAGGATCATTAACGAGTTAGGGTCTCTCCAGGCCCG
ACCTCCCAACCCTTTGTTTACCTTGCTGCATGTTGCTTTGGCGGACCCGTCTCGCGAC
CGCCCTGGGACTGCTGAGAGGCGTCTCTAGGCCCGTGTCCGCCGATAGCCAGCCTA
AACTCTTGGATGAATCGTGTATTATGTCTGAGTCTTGTGATTAAATCAAAAGCAAAAA
CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGCGAATTGCAGAATTCCCGTGAGTCATCGAATCTTTGAACGCACATTGCG
CCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTATCACCCCTCAAGCCCC
GTGCTTGGTGTTGGACGGATTTGGTCGTGTGATATCGACCCCTCCTAAAGACAATGA
CGGCGGCCTGCGGATCCCCCGGTACACTGAGCTTTTAACTGAGCACGTATCGGACAA
GGGCGCCCAGGACCCGGTCCTTCTCCTTTAACCAGGAAATTTCTTCAAGGTTGACCT
CGGATCAGGTAGGAATACGCGCTGAACTTAA

Acknowledgment

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The 32nd World Congress of Biomedical Laboratory Science (WMLS2018)

COI disclosure

I have no personal or financial interests to declare. I have no financial support from an industry source at the current presentation.

Microbiology

PA-90

Eukaryote-Made *Taq* Polymerase Enables Reliable Detection of Pathogens in Amniotic Fluid of Preterm Labor Cases

Development of a Novel Nested-PCR-Based Assay for Detecting *Mycoplasma*, *Ureaplasma*, other Bacteria and Fungi in Amniotic Fluid Samples

TOYAMA UNIVERSITY HOSPITAL

○ Tomohiro Ueno[1], Hideki Niimi[1], Noriko Yoneda[2], Satoshi Yoneda[2], Masashi Mori[3], Sigeru Saito[2] and Isao Kitajima[1]

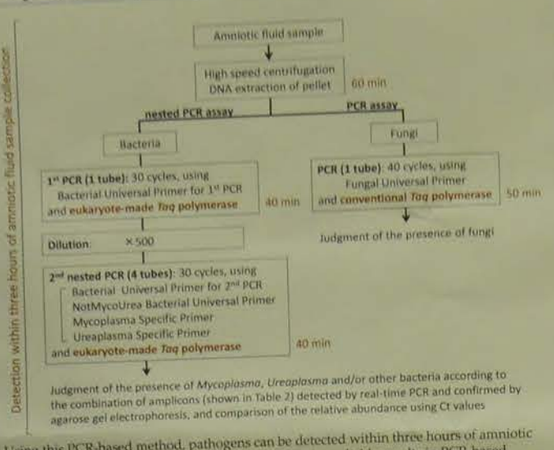
1. Clinical Laboratory Center, Toyama University Hospital, 2. Department of Obstetrics & Gynecology, Toyama University Hospital
3. Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University

Objectives

Intra-amniotic infection has long been recognized as the leading cause of preterm delivery. Microbial culture is the gold standard for the detection of intra-amniotic infection, but several days are required, and many bacterial species in the amniotic fluid are difficult to cultivate.

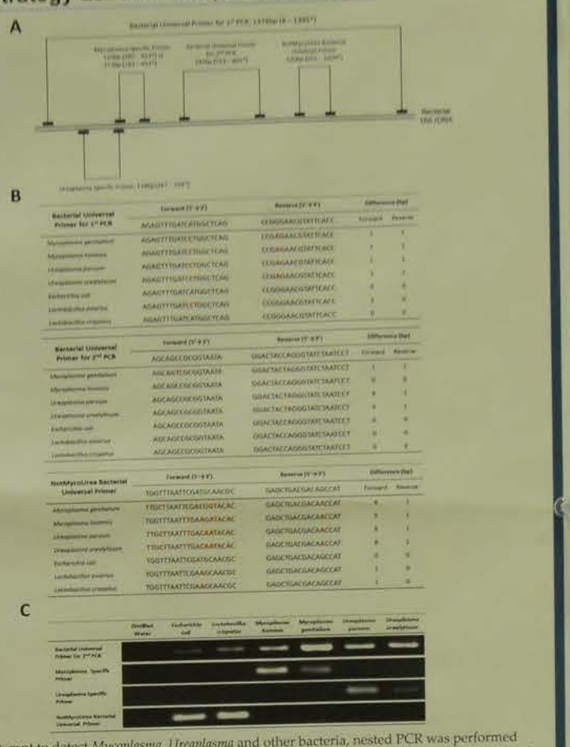
We developed a novel nested-PCR-based assay for detecting *Mycoplasma*, *Ureaplasma*, other bacteria and fungi in amniotic fluid samples within three hours of sample collection. To detect prokaryotes, eukaryote-made thermostable DNA polymerase, which is free from bacterial DNA contamination, is used in combination with bacterial universal primers. In contrast, to detect eukaryotes, conventional bacterially-made thermostable DNA polymerase is used in combination with fungal universal primers. To assess the validity of the PCR assay, we compared the PCR and conventional culture results using 300 amniotic fluid samples.

1. The workflow of the rapid detection method for *Mycoplasma*, *Ureaplasma*, other bacteria and fungi



Using this PCR-based method, pathogens can be detected within three hours of amniotic fluid sample collection. To prevent the occurrence of unreliable results in PCR-based assaying of amniotic samples for bacterial pathogens, eukaryote-made thermostable DNA polymerase (or *Taq* polymerase), which is free from bacterial DNA contamination, is used in combination with bacterial universal primers (along with two specific primers in the second, nested PCR). In contrast, for fungal pathogens, conventional bacterially-made thermostable DNA polymerase (or *Taq* polymerase), which is usually free from fungal DNA contamination, is used in combination with fungal universal primers.

2. The strategy used for the primer design



A) In an attempt to detect *Mycoplasma*, *Ureaplasma* and other bacteria, nested PCR was performed using the primer for the first PCR (Bacterial Universal Primer for 1st PCR) at the start. For the second (nested) PCR, four kinds of primers (Bacterial Universal Primer for 2nd PCR, Mycoplasma Specific Primer, Ureaplasma Specific Primer, and NotMycoUrea Bacterial Universal Primer) were used. *The amplicon sizes are described, and the amplified positons on *Escherichia coli* 16S ribosomal RNA are shown. Mycoplasma and Ureaplasma Specific Primers do not bind to *E. coli* 16S rDNA.
(**B**) The sequence homology between the primers (Bacterial Universal Primer for 1st PCR, Bacterial Universal Primer for 2nd PCR, NotMycoUrea Bacterial Universal Primer) and the target regions of *Mycoplasma*, *Ureaplasma* and other bacteria. Seven examples are shown as representative of *Mycoplasma*, *Ureaplasma* and other bacteria, respectively. The base sequence differences between the primers and the target regions are shown in red. Two of the primers (Bacterial Universal Primer for 1st PCR, Bacterial Universal Primer for 2nd PCR) can detect almost all kinds of bacteria including *Mycoplasma* and *Ureaplasma* species. On the other hand, the NotMycoUrea Bacterial Universal Primer can detect almost all kinds of bacteria, but does not detect *Mycoplasma* or *Ureaplasma* species because of the primer's low sequence homology, which is a key point of our method.
(**C**) The PCR amplification products of *Mycoplasma*, *Ureaplasma* and other bacteria amplified by each primer set. Six examples are used as representative of *Mycoplasma*, *Ureaplasma* and other bacteria, respectively. The gels showed no bacterial contamination using eukaryote-made thermostable DNA polymerase and also showed the specificity of each primer set. PCR amplification products were detected precisely according to the presence or absence of the targeted bacterial DNA templates.

3. Interpretation scheme for the absence or presence of *Mycoplasma*, *Ureaplasma*, and other bacteria in amniotic fluid samples

Bacterial Universal Primer for 2nd PCR	Mycoplasma Specific Primer	Ureaplasma Specific Primer	NotMycoUrea Bacterial Universal Primer	Interpretation of absence or presence
Amplicon size (bp) 287	Amplicon size (bp) 169 or 173	Amplicon size (bp) 128	Amplicon size (bp) 120	
–	–	–	–	None
+	+	–	–	*Mycoplasma*
+	–	+	–	*Ureaplasma*
+	–	–	+	Other bacteria
+	+	+	–	*Mycoplasma* and *Ureaplasma*
+	+	–	+	*Mycoplasma* and other bacteria
+	–	+	+	*Ureaplasma* and other bacteria
+	+	+	+	*Mycoplasma* and *Ureaplasma* and other bacteria

(+) detection of the amplicon,

(–) non-detection of the amplicon,

bp: base pairs

Other bacteria: bacteria other than *Mycoplasma* and *Ureaplasma*

4. The result of a comparison of the novel PCR and conventional culture results for detecting *Mycoplasma*, *Ureaplasma*, other bacteria and fungi

A *Mycoplasma*

Conventional culture method \ PCR method	+	–	Total
+	6	0	6
–	20	274	294
Total	26	274	300

B *Ureaplasma*

Conventional culture method \ PCR method	+	–*	Total
+	19	2*1	21
–	15	264	279
Total	34	266	300

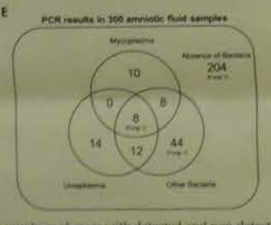
C Other Bacteria

Conventional culture method \ PCR method	+	–	Total
+	44	4*β	48
–	28	224	252
Total	72	228	300

D Fungi

Conventional culture method \ PCR method	+	–	Total
+	2	0	2
–	1	297	298
Total	3	297	300

E PCR results in 300 amniotic fluid samples



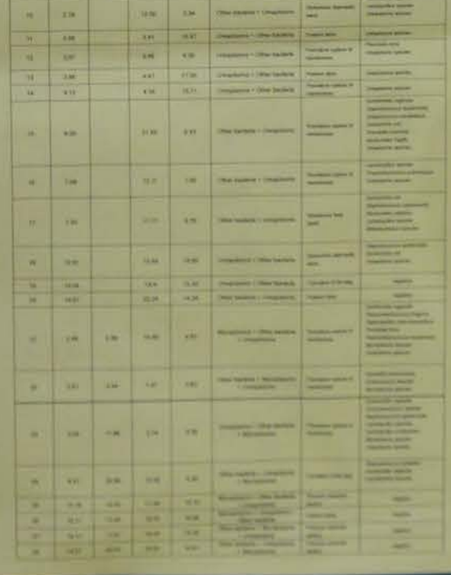
(A) The numbers of cases with detected and non-detected *Mycoplasma* in 300 amniotic fluid samples
(B) The numbers of cases with detected and non-detected *Ureaplasma* in 300 amniotic fluid samples
(C) The numbers of cases with detected and non-detected bacteria other than *Mycoplasma* and *Ureaplasma* in 300 amniotic fluid samples
(D) The numbers of cases with detected and non-detected fungi in 300 amniotic fluid samples
(E) PCR results in 300 amniotic fluid samples

Based on the detection level (positive and negative), 93.3% (280/300) of *Mycoplasma*, 94.3% (283/300) of the *Ureaplasma*, 89.3% (268/300) of other bacteria and 99.7% (299/300) of fungi results matched the culture results.

*α: Because of multiple colonies of other bacteria, colony-forming *Ureaplasma* could not be confirmed. In these cases, the existence of *Ureaplasma* was judged only by checking the color changes of the medium from yellow to red.

*β: Four samples were PCR-negative but culture-positive, and the bacteria detected by only the culture method were *Corynebacterium species* and *Lactobacillus species* (transvaginal amniocentesis), *Corynebacterium species* and *Lactobacillus species* (vaginal delivery), *Corynebacterium species* and *Lactobacillus species* (vaginal delivery) and *Lactobacillus species* (vaginal delivery).

5. The results of a comparison of the relative abundance of Mycoplasma, Ureaplasma, and/or other bacteria in amniotic fluid samples with mixed infection



Conclusions

Based on the detection level (positive and negative), 93.3% (280/300) of *Mycoplasma*, 94.3% (283/300) of *Ureaplasma*, 89.3% (268/300) of other bacteria and 99.7% (299/300) of fungi matched the culture results. Meanwhile, concerning the detection of bacteria other than *Mycoplasma* and *Ureaplasma*, 228 samples were negative according to the PCR method, 98.2% (224/228) of which were also negative based on the culture method. Employing the devised primer sets, mixed amniotic fluid infections of *Mycoplasma*, *Ureaplasma* and/or other bacteria could be clearly distinguished. In addition, we also attempted to compare the relative abundance in 28 amniotic fluid samples with mixed infection, and judged dominance by comparing the Ct values of quantitative real-time PCR.

We developed a novel PCR assay for the rapid detection of *Mycoplasma*, *Ureaplasma*, other bacteria and fungi in amniotic fluid samples. This assay can also be applied to accurately diagnose the absence of bacteria in samples. We believe that this assay will positively contribute to the treatment of intra-amniotic infection and the prevention of preterm delivery.

Microbiology

PA-91

Melting Temperature (Tm) Mapping Method:

A Novel Method for Rapid Identification of Unknown Pathogenic Microorganisms within Three Hours of Sample Collection

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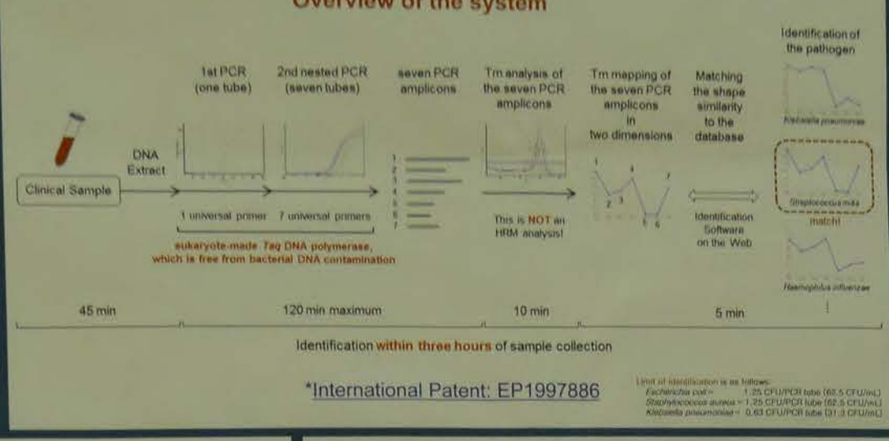
TOYAMA UNIVERSITY HOSPITAL

Objectives

As current blood culture methods require at least several days, empirically selected antibiotics are instead administered until the pathogenic microorganisms are identified. Though mass spectrometry can be utilized as a rapid identification method of pathogens, it requires the blood culture process, so it takes more than 10 hours (usually more than 24 hours) after samples are collected.

Our objective is to develop a novel rapid, easy, and cost-effective method (Tm mapping method) which enables identification of pathogenic microorganisms within three hours after patient sample collection.

1. A novel rapid system that identifies unknown pathogenic microorganisms

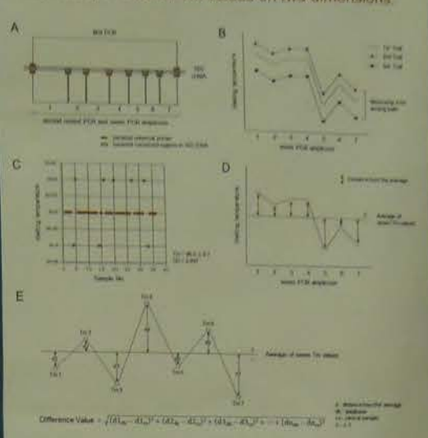


*International Patent: EP1997886

Limit of identification is as follows:
Escherichia coli = 1.25 CFU/PCR tube (62.5 CFU/mL)
Staphylococcus aureus = 1.25 CFU/PCR tube (62.5 CFU/mL)
Klebsiella pneumoniae = 0.63 CFU/PCR tube (31.3 CFU/mL)

2. The Tm mapping method

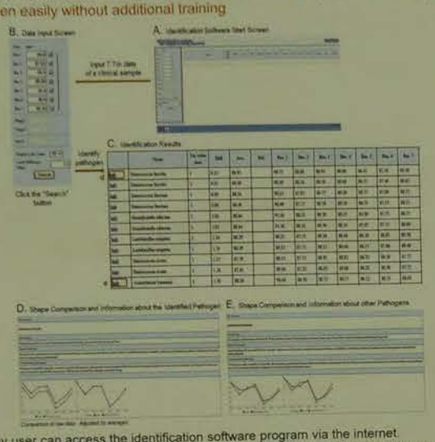
The Tm mapping method is a novel method that identifies pathogenic microorganisms by mapping the unique shape of seven Tm values on two dimensions.



(**A**) The strategy of primer designs is shown. Nested PCR is performed using seven bacterial universal primer sets, and then the seven Tm values, which reflect the different DNA base sequences, are obtained.
(**B**) Mapping the seven Tm values on two dimensions leads to the identification of the unique shape of the pathogen, like a constellation. The average of all the seven Tm values reflects the measurement error among trials, but the Tm mapping shape is not affected by this kind of error.
(**C**) Using an analytical instrument with a high degree of thermal accuracy among PCR tubes, and Tm value analysis with EvaGreen dye on 36 samples of the same bacterial DNA in the same trial, the tube-to-tube variation was within ±0.1℃, which has little effect on the Tm mapping shape of the pathogen.
(**D**) The distance of each individual Tm value from the average (X-axis) is not affected by the measurement error among trials, which means that the shape does not change. The Tm mapping shape is identified by comparing the seven distances obtained from the unknown bacteria to those in the database.
(**E**) To identify a pathogen, the identification software program calculates the **Difference Values** using the indicated formula. The closer the Difference Value is to zero, the more similar the Tm mapping shape is to the shape of a pathogenic bacteria in the database.

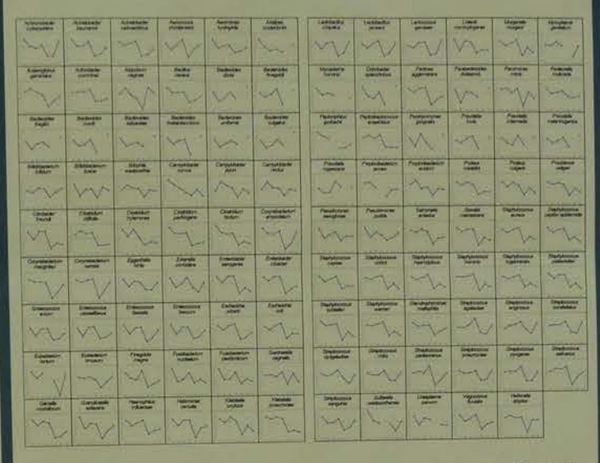
3. The Identification software on the web

Using the identification software program on the web, any user can identify the pathogen easily without additional training



(**A**) Any user can access the identification software program via the internet.
(**B**) First, the user input the seven Tm value data of a clinical sample, and clicks the "search" button.
(**C**) The identification results are shown promptly. The results are lined up in ascending order of the Difference Values.
(**D**) Next, the user clicks the "Info" button where information about the pathogen can be seen. It is also possible to compare the raw data to the Tm mapping shape in the database.
(**E**) Information about other pathogens can be seen as well, and the difference between the shapes can be seen.

4. The Tm mapping shapes of bacteria registered in the database



107 kinds of bacteria

* The X-axis is the average of the seven Tm values.

5. The Interpretative Criteria

This table shows the interpretative criteria for identification based on the **Difference Values**.

Difference Value (D)	Suitability for identification	Identification	Interpretation
0.0 ≤ D ≤ 0.28	High	All identification results within this range have the same possibility of being the bacterial isolate	Matched the bacteria registered in the database
0.28 < D ≤ 0.5	Medium to Low	The identification result with the lowest Difference Value is highly likely to be the bacterial isolate	Mutant strain, or Polymicrobial infection*1
0.5 < D	Not suitable	Does NOT identify the bacterial isolate	Polymicrobial infection*2, or Not registered in the database, or Under the limit of identification*3

*1: Polymicrobial infection with one dominant bacterial species. *2: Polymicrobial infection with no dominant bacterial species.
*3: Under the limit of identification, but over or equal to the limit of detection.

6. Comparison of the Tm mapping and culture results starting from whole blood samples

Bacterial isolates		Tm mapping method		
	detection	+	−	Total
Conventional culture method	+	43[A] (I: 41, NS: 2)	2[C]	45
	−	27[B] (I: 18, NS: 9)	128[D]	155
	Total	70 (I: 59, NS: 11)	130	200

I: Identified according to the Tm mapping method (Difference Value ≤ 0.5)
NS: Bacteria were detected, but not suitable for Tm mapping identification (Difference Value > 0.5)
A: 41 Tm mapping identifications matched the culture (or sequencing) results, whereas two samples were not suitable for Tm mapping identification due to the presence of polymicrobial infection.
B: 18 species could be identified and nine samples were not suitable for Tm mapping identification.
C: These two samples were positive for *Staphylococcus epidermidis* and *Bacillus cereus*.
D: These samples were negative using both the Tm mapping method and culture method.

7. Individual results of identification starting from patient samples

Tm mapping method	Diff.	Identification results: Conventional culture method	Identification results: Sequencing method	Match: Cult.	Match: Seq.	Comments
Control: Healthy whole blood						
None detected	–	No culture growth		–	–	Negative control
Patient						
1	Staphylococcus capitis / epidermidis	0.09	Staphylococcus epidermidis		✔	
2	Staphylococcus capitis / epidermidis	0.12	Staphylococcus epidermidis		✔	
3	Staphylococcus capitis / epidermidis	0.13	No culture growth	Staphylococcus epidermidis	–	✔
4	Streptococcus agalactiae	0.13	Streptococcus agalactiae	Streptococcus agalactiae	✔	✔ dominant bacteria
5	Staphylococcus aureus	0.14	Staphylococcus aureus		✔	
6	Staphylococcus capitis / epidermidis	0.15	Staphylococcus epidermidis		✔	
7	Staphylococcus haemolyticus	0.15	Staphylococcus haemolyticus		✔	
8	Bacteroides vulgatus	0.15	Bacteroides vulgatus			✔
9	Acinetobacter baumannii	0.16	No culture growth	Acinetobacter baumannii		✔
10	Staphylococcus aureus	0.16	Staphylococcus aureus		✔	
11	Escherichia coli	0.16	No culture growth	Escherichia coli	–	✔
12	Escherichia coli	0.16	Escherichia coli		✔	
13	Bacillus cereus	0.17	No culture growth	Bacillus cereus	–	✔
14	Staphylococcus haemolyticus	0.17	Staphylococcus haemolyticus		✔	
15	Escherichia coli	0.18	No culture growth	Escherichia coli	–	✔
16	Staphylococcus capitis / epidermidis	0.18	No culture growth	Staphylococcus epidermidis	–	✔
17	Streptococcus pyogenes	0.18	Streptococcus pyogenes	Streptococcus pyogenes	✔	✔
18	Staphylococcus capitis / epidermidis	0.18	Staphylococcus epidermidis		✔	
19	Escherichia coli	0.18	Escherichia coli		✔	
20	Staphylococcus caprae	0.19	Staphylococcus epidermidis	Staphylococcus caprae	✔	✔
21	Enterococcus faecium	0.19	No culture growth	Enterococcus faecium	–	✔
22	Staphylococcus aureus	0.19	No culture growth	Staphylococcus aureus	–	✔
23	Staphylococcus aureus	0.19	Staphylococcus aureus		✔	
24	Staphylococcus capitis / epidermidis	0.19	Staphylococcus epidermidis		✔	
25	Staphylococcus caprae	0.20	Staphylococcus epidermidis	Staphylococcus caprae	✔	✔
26	Pseudomonas aeruginosa	0.20	Pseudomonas aeruginosa		✔	
27	Bacillus cereus	0.20	Bacillus cereus		✔	
28	Staphylococcus haemolyticus	0.21	Staphylococcus aureus	Staphylococcus haemolyticus	✔	✔
29	Escherichia coli	0.21	Escherichia coli Bacteroides thetaiotaomicron Bacteroides vulgatus	Escherichia coli	✔	✔ dominant bacteria
30	Escherichia coli	0.21	Escherichia coli		✔	
31	Staphylococcus haemolyticus	0.22	No culture growth	Staphylococcus haemolyticus	–	✔
32	Staphylococcus capitis / epidermidis	0.22	Staphylococcus epidermidis		✔	
33	Staphylococcus caprae	0.22	Staphylococcus epidermidis	Staphylococcus caprae	–	✔
34	Staphylococcus haemolyticus or Staphylococcus lugdunensis	0.23 0.26	Staphylococcus haemolyticus	Staphylococcus haemolyticus	✔	✔ Similar match
35	Staphylococcus caprae	0.24	No culture growth	Staphylococcus caprae	–	✔
36	Staphylococcus aureus	0.24	No culture growth	Staphylococcus aureus	–	✔
37	Staphylococcus capitis / epidermidis	0.24	Staphylococcus epidermidis			✔
38	Escherichia coli	0.25	No culture growth	Escherichia coli	✔	
39	Klebsiella pneumoniae	0.25	Klebsiella pneumoniae		✔	
40	Staphylococcus capitis / epidermidis	0.26	Staphylococcus epidermidis		✔	
41	Escherichia coli	0.26	Escherichia coli		✔	
42	Citrobacter freundii	0.26	Citrobacter freundii		✔	
43	Staphylococcus caprae	0.27	Staphylococcus epidermidis	Staphylococcus caprae	–	✔
44	Acinetobacter calcoaceticus	0.28	No culture growth	Acinetobacter calcoaceticus	–	✔
45	Escherichia coli	0.28	Escherichia coli Enterococcus faecalis	Escherichia coli	✔	✔ dominant bacteria
46	Staphylococcus hominis	0.28	Staphylococcus hominis			✔
47	Escherichia coli	0.30	No culture growth	Escherichia coli	✔	
48	Escherichia coli	0.30	Escherichia coli		✔	
49	Klebsiella pneumoniae	0.30	Klebsiella pneumoniae			✔
50	Staphylococcus lugdunensis	0.31	No culture growth	Staphylococcus lugdunensis	✔	
51	Klebsiella pneumoniae	0.31	Klebsiella pneumoniae		✔	
52	Staphylococcus capitis / epidermidis	0.31	Staphylococcus epidermidis		✔	
53	Staphylococcus warneri	0.32	No culture growth	Staphylococcus warneri	–	✔
54	Enterococcus faecalis	0.32	Enterococcus faecalis		✔	
55	Escherichia coli	0.32	No culture growth	Escherichia coli	–	✔
56	Enterococcus faecalis	0.33	No culture growth	Enterococcus faecalis	–	✔
57	Escherichia coli	0.33	No culture growth	Escherichia coli	–	✔
58	Escherichia coli	0.34	No culture growth	Escherichia coli	–	✔
59	Staphylococcus hominis	0.34	No culture growth	Staphylococcus hominis	–	✔
60	Escherichia coli	0.34	No culture growth	Escherichia coli	–	✔
61	Escherichia coli	0.34	No culture growth	Escherichia coli	–	✔
62	Klebsiella pneumoniae	0.35	No culture growth	Klebsiella pneumoniae	–	✔
63	Escherichia coli	0.35	No culture growth	Escherichia coli	–	✔
64	Escherichia coli	0.36	No culture growth	Escherichia coli	–	✔
65	Escherichia coli	0.36	No culture growth	Escherichia coli	–	✔
66	Staphylococcus hominis	0.36	Staphylococcus hominis			✔
67	Escherichia coli	0.37	No culture growth	Escherichia coli	✔	
68	Escherichia coli	0.37	Escherichia coli		✔	
69	Klebsiella pneumoniae	0.37	Klebsiella pneumoniae			✔
70	Staphylococcus lugdunensis	0.38	No culture growth	Staphylococcus lugdunensis	✔	
71	Klebsiella pneumoniae	0.38	Klebsiella pneumoniae		✔	
72	Staphylococcus capitis / epidermidis	0.38	Staphylococcus epidermidis		✔	
73	Staphylococcus warneri	0.38	No culture growth	Staphylococcus warneri	–	✔
74	Escherichia coli	0.38	Escherichia coli		✔	
75	Escherichia coli	0.39	No culture growth	Escherichia coli	–	✔
76	Escherichia coli	0.39	Escherichia coli		✔	
77	Staphylococcus hominis	0.39	Staphylococcus hominis			✔
78	Escherichia coli	0.40	No culture growth	Escherichia coli	✔	
79	Escherichia coli	0.40	Escherichia coli		✔	
80	Klebsiella pneumoniae	0.40	Klebsiella pneumoniae			✔
81	Klebsiella pneumoniae	0.41	No culture growth	Klebsiella pneumoniae		✔
82	Staphylococcus capitis / epidermidis	0.41	No culture growth	Staphylococcus epidermidis		✔
83	Staphylococcus warneri	0.42	No culture growth	Staphylococcus warneri		✔
84	Escherichia coli	0.42	No culture growth	Escherichia coli		✔
85	Escherichia coli	0.42	No culture growth	Escherichia coli		✔
86	Staphylococcus hominis	0.43	No culture growth	Staphylococcus hominis	✔	✔

Diff. = Difference Value

Cult. = Culture results

Seq. = Sequencing results

DB = Database

✔: matched with the culture/sequencing result

×: did not match with the culture/sequencing result

—: did not perform a comparison with the culture/sequencing result

The bold line in the Difference Value column marks the interpretative criteria boundary (0.28) described in Table 6.

Conclusions

The Tm mapping method is rapid and accurate, and would contribute to the rescue of patients with severe infections, as well as a decrease in the development of antibiotic resistance.

Characteristics of the novel system are as follows.

- The novel system can rapidly identify the dominant bacteria in a patient sample by mapping the unique shape of seven Tm values on two dimensions.
- Using only seven primer sets, more than 100 bacterial species can be identified without sequencing, and the number of identifiable bacterial species is easily expandable.
- The expected cost of the new system is very low in comparison to the cost of the conventional method, resulting in a major cost reduction.
- Without additional training, any user can identify the bacteria species easily using the identification software program on the World Wide Web.

PA-92

Evaluating the Usability of a New Rapid Immunoassay Test Kit for Detecting Urinary Tract Infections

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The 32nd World Congress of Biomedical Laboratory Science
International Innovation of Laboratory Medicine —Basic and Advanced—

TEIKYO

50th

Abstract

INTRODUCTION Currently, there are no commercially available test kits in Japan to detect urinary tract infections (UTIs) rapidly. A rapid immunoassay test for detecting UTIs called 'RapidBac' was developed by Silver Lake Research Corporation, USA. We tested if the RapidBac is effective in detecting UTIs.

METHODS We tested the types of bacteria detectable by RapidBac using 23 ATCC standard strains and clinical isolated strains (gram-negative bacteria n=14, gram-positive bacteria n=8, Yeast n=1). To determine the minimum sensitivity of RapidBac, we used 5 different concentrations (10^5, 10^4, 10^3, 10^2, 10 CFU/mL) of *Escherichia coli* (ATCC 35218). We also tested and compared RapidBac using 30 urine specimens that were submitted to the Teikyo University Hospital for urine cultures.

RESULTS Yeast and all gram-positive bacteria tested negative. Of the 14 gram-negative bacteria, 9 strains tested positive and 5 strains tested negative. The negative strains include *Morganella morganii*, *Proteus stuartii*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Serratia liquefaciens*. The RapidBac kit showed a pale test line on samples with concentrations ≤ 10^3 CFU/mL, indicating a positive but weak result. However for samples with concentrations ≥ 10^4 CFU/mL, the test line was clearly observed and the test results were considered positive. The sensitivity and specificity were 76.5% and 84.6% respectively. Among 30 urine specimens, only 2 specimens showed a false negative (15.4%) result, both including *Pseudomonas aeruginosa*. The false positive rate was 23.5%.

CONCLUSIONS The RapidBac detected gram-negative bacteria accurately in this study, except some strains from *Pseudomonas* species. We think the low detection rate of *Pseudomonas* species, the main pathogenic bacterium for complicated UTIs, could be a problem and needs further investigation. We also think that the RapidBac would be an efficient tool for hospitals without bacteriological tests or as a point-of-care-testing kit.

Purpose

Currently, Gram staining and urine cultures are the main methods to detect bacteria from a urinary tract infection (UTI) specimen. However, it takes 24 to 48 h to report the species and the amount of the pathogenic bacteria. Furthermore, results are not readily available to patients, often needing an extra visit to the hospital.

For a rapid test, a simple Gram staining might be effective just to know if there is any bacterial population in the urine and can be done in less than 30 min; however we cannot count the amount and type of existing bacteria. Therefore, a confirmed diagnosis of a UTI cannot be made. Furthermore, facilities without a microbiology testing room such as clinics cannot even examine UTIs, when needed. Lack of any commercially available rapid UTIs test kit makes these problems common in Japan.

A rapid immunoassay test, UTIs called 'RapidBac' for detecting was developed by Silver Lake Research Corporation, USA. In this study, we evaluated the usability of RapidBac for detecting UTIs.

RapidBac™

The RapidBac test is a lateral flow immunoassay test strip, in which monoclonal antibodies specific for bacterial cell surface determinants bind to the target bacterium in a "sandwich" manner, producing a visual signal when bacteria are present.

Kit Components



Figure 1. Kit Components

- Assay Buffer
- Test Strip
- Test Vial
- 2 Volumetric Pipettes

Add assay buffer (2 min.)
↓
Add urine (5 min.)
↓
Place test strip in the test vial (10 min.)
↓
Remove strip and read the result (Total 17min.)

Results

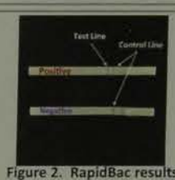


Figure 2. RapidBac results

Materials & Methods

[1] Sensitivity
We examined the sensitivity of the RapidBac kit using *Escherichia coli* American Type Culture Collection (ATCC) 35218 strain. The concentrations were adjusted to 10^8 (McFarland No. 0.5), 10^5, 10^4, 10^3, 10^2, and 10 CFU/mL.

[2] Detectable Bacteria
We examined 23 bacterial strains (ATCC n=18, Clinical Isolates n=5). This included bacteria that cause UTIs or normal bacteria that might contaminate urine (gram-negative rods (GNR) n=14, gram positive (GP) n=8, Yeast n=1). The concentration was adjusted to 1.0×10^5CFU/mL.

[3] Clinical Specimens
Thirty urine specimens collected at the Teikyo University Hospital between 4/2/2016 and 4/12/2016 were examined. The results from the Gram stains, cultures and RapidBac were compared. For the patient's background, gender, age, basic disease, usage of antibiotics, and urinalysis were investigated.

[4] Sensitivity for *Pseudomonas aeruginosa*
The sensitivity of RapidBac for *P. aeruginosa* was examined using an isolated strain(No. 31). The concentrations were adjusted to 10^8(McFarland No. 0.5), 10^7, 10^6, 10^5, 10^4, and 10^3 CFU/mL

[5] Detectable Conditions (Autoclave)
To evaluate whether dead bacteria would be detectable, we examined *E. coli* (ATCC 35218) after autoclave sterilization (2 atm, 121°C, 20min). The concentration was adjusted to McFarland No. 0.5.

[6] Detectable Conditions (Antibiotics)
To examine the detection of bacteria under an antibiotic-used condition, we incubated 1.0×10^5 CFU/mL *E. coli* (ATCC 35218) with either 1 μg/mL or 10 μg/mL of Cefotaxime (CTX) for 1h, 3h, and 24h.

*All tests were performed according by the instructions of this kit.
CFU = Colony forming units

Results

[1] Sensitivity

Table 1. Minimum sensitivity

CFU/mL	Test Line	Results
McFarland 0.5	+	Positive
1.6×10^4	+	Positive
2.5×10^3	W+	Positive
1.5×10^3	−	Negative
2.0×10^1	−	Negative
1.0×10^1	−	Negative
Negative Control	−	Negative

Minimum sensitivity: 10^4 CFU/mL

At 10^3 CFU/mL, there were few tests that showed negative results and pale test lines. Therefore, the minimum sensitivity of the kit was determined to be 10^4 CFU/mL, the concentration that clear positive results.

*W+ indicates that the test line was pale.

[2] Detectable bacteria

Table 2. Detectable bacteria

GNR	Results	GPC	Results
Acinetobacter baumanni	+	*Enterococcus faecalis*	−
Citrobacter freundi	+	*Enterococcus faecium*	−
Escherichia coli	+	*Enterococcus casseliflavus*	−
Enterobacter cloacae	+	*Staphylococcus aureus*	−
Enterobacter aerogenes	+	*Staphylococcus epidermidis*	−
Klebsiella pneumoniae	+	*Staphylococcus capitis*	−
Klebsiella oxytoca	+	*Staphylococcus haemolyticus*	−
Proteus mirabilis	+		
Proteus stuartii	+	GPR	
Stenotrophomonas maltophilia	+	*Corynebacterium* spp.	−
Morganella morganii	−		
Pseudomonas aeruginosa	−	Yeast	
Pseudomonas fluorescens	−	*Candida albicans*	−
Serratia liquefaciens	−		

As shown in Table 2, 9 species of GNRs were detectable.
All gram-positive bacteria and yeast were not detectable.
From the results, RapidBac has the ability to detect *Enterobacteriaceae* mainly. It does not react to bacteria likely to contaminate the urine when collected.

Patient Background

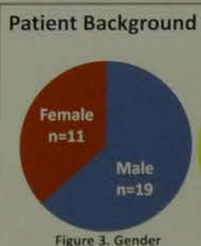


Figure 3. Gender

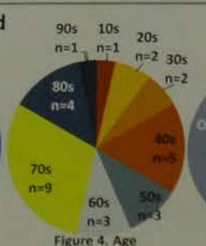


Figure 4. Age

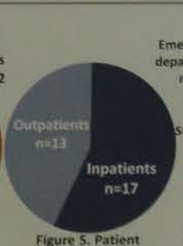


Figure 5. Patient



Figure 6. Clinical departments

Table 3. Usage of antibiotics

Antibiotics	Usage
Penicillins	n = 1
1st generation Cefalosporins	n = 2
3rd generation Cefalosporins	n = 2
β-lactamase inhibitors	n = 5
Carbapenems	n = 2
Glycopeptides	n = 4
Aminoglycosides	n = 1
Macrolides	n = 1
Tetracyclines	n = 1
New quinolones	n = 4
Sulfamethoxazole trimethoprim	n = 2

Table 4. Urine pH

mean	6.5
SD	0.99

Table 5. Urine specific gravity

mean	1.017
SD	0.0059

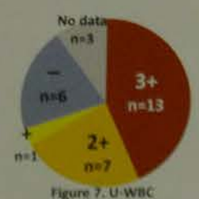


Figure 7. U-WBC

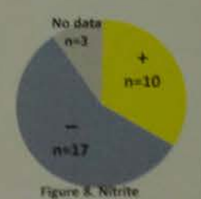


Figure 8. Nitrite

[3] Clinical specimens

[False Positive]

Test lines for sample No. 4, 15, and 28 were pale, but we could not analyze the cause. Besides these three, the test line for specimen No. 25 was needed to be seen clearly. In the gram-positive strain specimen, a gram-negative rod was seen, but it did not grow when incubated under gram-negative conditions.

GNR (10^4 cfu/mL)	RapidBac +	RapidBac −	Total
+	13 (76.5%)	2 (15.4%)	15
−	4 (23.5%)	11 (84.6%)	15
Total	17	13	30

24 out of 30 specimens' results matched
Sensitivity: 76.5% Specificity: 84.6%
4 specimens resulted in false positive
and 2 specimens in false negative

[False Negative]

P. aeruginosa was isolated from the two urine specimens (No. 6 and 29). Initially we thought that RapidBac could not detect this bacterium, as shown in [2], but *P. aeruginosa* was detected in specimen No.27, Table 5.
Therefore, we focused on the amount of bacteria in the urine.

[5] Detectable conditions (autoclave)

The results were positive even after autoclave sterilization. Gram staining showed light-stained bacterial cells, indicating that the test might react if dead bacterial cells remained in the urine.

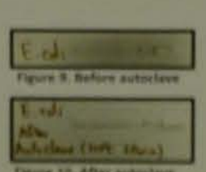


Figure 9. Before autoclave

Figure 10. After autoclave

[6] Detectable conditions (antibiotics)

Cefotaxime	Incubation Time	RapidBac Results
1 μg/mL	1 hour	Positive
10 μg/mL	3 hours	Positive
1 μg/mL	24 hours	Positive
10 μg/mL	24 hours	Positive

Under all tested conditions, the results were positive. This indicates that bacteria would be detectable by RapidBac even when antibiotics are metabolized in the urine.

We examined 4 additional urine specimens that contained *P. aeruginosa* only. As seen in Table 9, RapidBac detected *P. aeruginosa* at the concentration near 10^7 CFU/mL.

One of the specimens contained 10^3 CFU/mL and tested negative. *P. aeruginosa* needs a high concentration to be detected.

[4] Sensitivity for *P. aeruginosa*

Minimum Sensitivity: 10^7 CFU/mL

At 10^7 CFU/mL, P. aeruginosa was detected properly. This indicates that the negatively tested GNRs at [2] might become positive at a higher concentration.

Conclusions

We think RapidBac would be a useful tool to detect uncomplicated UTIs for its detectable range of bacteria, specificity, and the sensitivity that meets the standard of UTIs. RapidBac can also be used for patients with different backgrounds.

Although RapidBac can detect uncomplicated UTIs, its ability to detect complicated UTIs is relatively weaker because of its low sensitivity for *P. aeruginosa*, the main pathogenic bacterium of complicated UTIs and the cause of false negative results in this study.

Although we could not investigate the main cause of the false positive results, we think that there is a strong possibility of a non-specific reaction. As we investigated further, we discovered that RapidBac detects dead bacteria too. This indicates that the test results might appear positive when the patient is being treated for an UTI and the antibiotics are metabolized in his/her urine.

Finally, since RapidBac only takes approximately 20 min in total, the patient can know the results during their single visit to the hospital. Furthermore, as the instructions are simple and easy to use, RapidBac can be used not only in hospitals but also in facilities without a microbiological testing room, such as clinics or even as a point-of-care testing.

Microbiology

PA-93

Identification and antimicrobial susceptibilities in non-beta hemolytic streptococci causing bloodstream infections

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Background/introduction

Non-beta hemolytic streptococci (mostly viridans streptococci) are normal flora in oral cavities (normal oral commensals). Although they have been considered to be of low virulence, they can cause bacteremia and sometimes infective endocarditis in certain individuals with underlying diseases such as cardiac valve disease. Identification of non-beta hemolytic streptococci has been challenging in routine clinical microbiology testing since conventional biochemical methods do not accurately discriminate and identify these species. In this study, we compared the identification results of conventional biochemical tests with those of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and evaluated the antimicrobial susceptibilities which is important in choosing antibiotics in severe infections.

Materials/Methods

The 54 blood culture isolates analyzed in this study were detected as suspected non-beta hemolytic streptococci (excluding *Streptococcus pneumoniae*) by conventional methods (VITEK2 and Rapid ID32) in Nara Medical University hospital between 2009 and 2015. Identification was performed with both VITEK MS (bioMérieux) and MALDI Biotyper (Burker). Susceptibility tests were performed by Dry-plate Eiken DP34 (Eiken) for 52 isolates identified as viridans streptococci with MALDI-TOF.

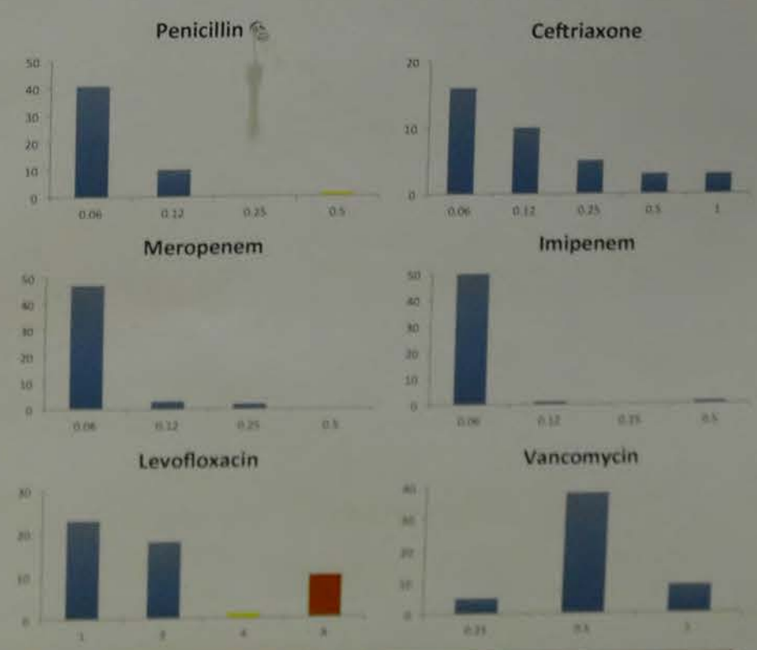
Discussion

- The three methods showed full agreement for 27 isolates (50.0%).
- The agreement rate between VITEK MS and MALDI Biotyper was 51 isolates (94.4%).
- Even for the isolates that were not detected to species level by conventional methods, VITEK MS and MALDI Biotyper showed full agreement.
- Erroneous identification of *S. mitis* as *S. pneumoniae* by MALDI-TOF has been reprted[1].
- Based on the identification with MALDI-TOF, *Streptoccocus mitis/oralis* were the most common species (37.0%) followed by *Streptococcus anginosus* and *Streptococcus constellatus* (11.1%), and *Streptococcus parasanguinis* and *Streptococcus salivarius* (7.4%).
- Susceptibilities to penicillin G, ceftriaxone, levofloxacin, vancomycin, imipenem and meropenem were 98.1, 100, 78.8, 100, 100, and 100%, respectively.

1. J Clin Microbiol 2012;50:2863

Results

Conventional method	VITEK MS	MALDI Biotyper
Streptococcus mitis/Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus mitis/Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus mitis/Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus infantis*
Streptococcus mitis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus mitis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus mitis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus pneumoniae*
Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus oralis	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus gordonii	*Streptococcus gordonii*	*Streptococcus mitis*
Streptococcus gordonii	*Streptococcus gordonii*	*Streptococcus gordonii*
Streptococcus sanguinis	*Streptococcus sanguinis*	*Streptococcus gordonii*
Streptococcus sanguinis	*Streptococcus sanguinis*	*Streptococcus sanguinis*
Streptococcus parasanguis	*Streptococcus parasanguinis*	*Streptococcus sanguinis*
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus mitis*
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus anginosus	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus anginosus	*Streptococcus gallolyticus* subsp *pasteurianus*	*Streptococcus gallolyticus*
Streptococcus anginosus	*Streptococcus parasanguinis*	*Streptococcus parasanguinis*
Streptococcus constellatus	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus constellatus	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus constellatus	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus constellatus	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus intermedius	*Streptococcus intermedius*	*Streptococcus intermedius*
Streptococcus intermedius	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus intermedius	*Streptococcus gallolyticus* subsp *pasteurianus*	*Streptococcus gallolyticus*
Streptococcus salivarius	*Streptococcus salivarius* subsp *salivarius*	*Streptococcus salivarius*
Streptococcus salivarius	*Streptococcus salivarius* subsp *salivarius*	*Streptococcus salivarius*
Streptococcus salivarius	*Streptococcus salivarius* subsp *salivarius*	*Streptococcus salivarius*
Streptococcus salivarius	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus mutans	*Streptococcus mutans*	*Streptococcus mutans*
Granulicatella adiacens	*Granulicatella adiacens*	*Granulicatella adiacens*
Granulicatella adiacens	*Granulicatella adiacens*	*Granulicatella adiacens*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus viridans group	*Streptococcus gordonii*	*Streptococcus gordonii*
Streptococcus viridans group	*Streptococcus infantarius* subsp *coli* (*S. lutetiensis*)	*Streptococcus lutetiensis*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus viridans group	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
Streptococcus viridans group	*Streptococcus parasanguinis*	*Streptococcus parasanguinis*
Streptococcus milleri group	*Streptococcus constellatus*	*Streptococcus constellatus*
Streptococcus Group F	*Streptococcus anginosus*	*Streptococcus anginosus*
Streptococcus spp.	*Streptococcus infantarius* subsp *coli* (*S. lutetiensis*)	*Streptococcus lutetiensis*
Streptococcus spp.	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus mitis*
α-Streptococcus	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
α-Streptococcus	*Streptococcus mitis/Streptococcus oralis*	*Streptococcus oralis*
α-Streptococcus	*Streptococcus parasanguinis*	*Streptococcus parasanguinis*
γ-Streptococcus	*Streptococcus gallolyticus* subsp *pasteurianus*	*Streptococcus gallolyticus*
γ-Streptococcus	*Streptococcus salivarius* subsp *salivarius*	*Streptococcus salivarius*



Conclusion

Identification of non-beta hemolytic streptococci causing bloodstream infections by MALDI-TOF is rapid, cost effective and accurate and may be a better alternative to conventional methods. Resistance to levofloxacin should be taken into account in selecting antibiotics.

Microbiology

PA-94

VOLATILE METABOLITES AS EARLY DIAGNOSTICS FOR URINARY TRACT INFECTIONS?

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INTRODUCTION

Urinary tract infections (UTI) pose serious health problems throughout the world affecting millions of people each year. UTIs rank second only to respiratory infections in the United States and account for about 9.6 million doctor visits annually. However, many of the urine samples that are received by the clinical laboratories may contain contaminants only and very few or no infecting microbes. The current techniques that are used for the detection and identification of micro-organisms include microscopy, growing different cultures, and more recently DNA based methods such as PCR. Though these methods are the most preferred ones, their main disadvantage is the slow speed of analysis.

In contrast, selected ion flow tube mass spectrometry (SIFT-MS) simultaneously detects and quantifies volatile organic compounds (VOCs) in real time that are produced by the infecting organisms to pptv levels with no sample preparation.[1,2] In this way, if different organisms produce unique types of compounds then monitoring those compounds aids the detection and identification of the infection.

We show here how readily a SIFT-MS instrument can be applied to measure the microbial VOCs (MVOCs) occurring in the headspace of urine samples inoculated with UTI-causing microbes such as *E. coli, P. vulgaris, P. aeruginosa, S. aureus, S. epidermidis, C. albicans* etc.

METHOD

SIFT-MS uses ultra-soft, precisely controlled chemical ionization coupled with mass spectrometric detection (Figure 1) to rapidly detect and quantify VOCs and inorganic gases to low part-per-trillion concentrations by volume (pptv).[1]

Eight different microbial species were used in the study (10 replicate samples of each). The samples were prepared using thawed sterile urine (20 mL) from healthy males inoculated to a concentration of between 107 and 109 cfu/mL and incubated at 37°C for 6 h. The headspace of each urine sample was then tested through the sample inlet of a Voice200. Each sample was assayed using SIFT-MS in SIM scan mode for 45 seconds.

Each VOC was monitored using product ion masses that were selected to avoid masses that would overlap with other known compounds. Because the reaction rates between the analytes and the reagent ions are known, quantification is achieved in near-real time using on-board software and the in-built library.

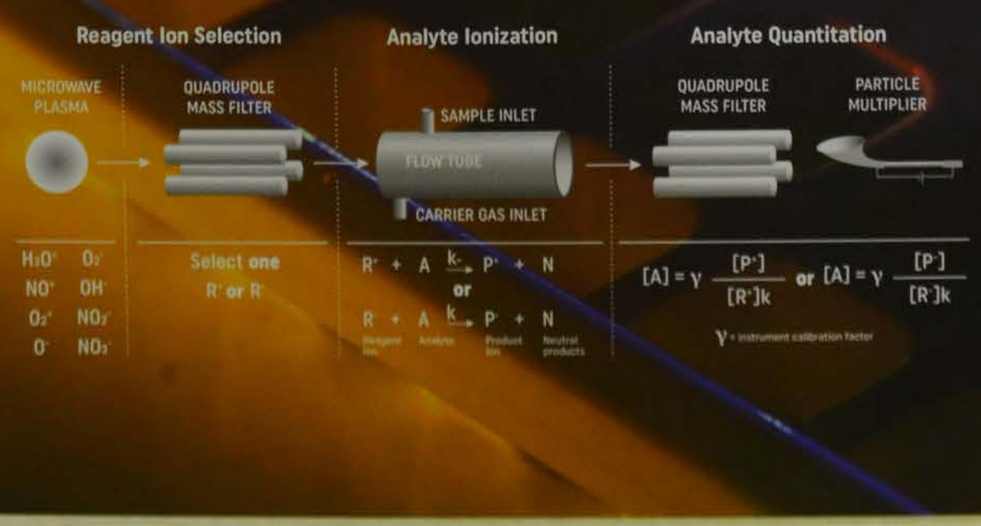
RESULTS AND DISCUSSION

The approximate microbe concentration was determined by comparing the standard bacterial growth curves (optical density measurements at 580 nm and 686 nm). Spread plates prepared from the inoculated urine showed very heavy but homogenous growth after 24 hour incubation period indicating inoculation of single bacterial strain. The blank urine control plates showed no growth after 24 hour incubation indicating no contamination from external sources.

Figure 2 shows the sample MVOC mean concentrations minus the mean blank/negative controls + 2 standard deviations detected using SIFT-MS technique. Negative values show the microorganism has used the compound. These data illustrate (i) the simplicity with which SIFT-MS can be applied to profiling chemically diverse volatile compounds in a single analysis, and (ii) the differentiation of different strains of microorganisms, which enables detection and identification of the microorganisms associated with different types of UTIs.

Despite having differences in culture media and sampling procedures there was some agreement in the current finding and previous studies. For example, *S. aureus* produced significant amounts of ammonia and dimethyl disulfide,[3] *P. vulgaris* produced methyl mercaptan,[4] production of indole indicated *E.coli*.[5] However, there was no significant increase in 2-aminoacetophenone which was found to indicate the presence of *P. aeruginosa*. A possible reason is that earlier studies used different culture media and a different analysis technique.

Figure 1: A schematic representation of dual-polarity SIFT-MS.



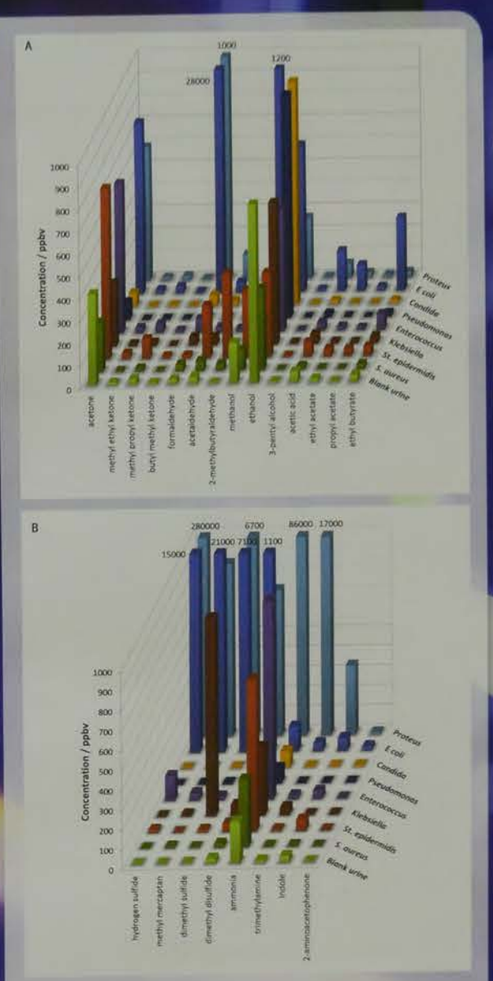


Figure 2. Profiles of volatile compounds produced by the UTI-causing microorganisms detected using SIFT-MS: (a) oxygenates and (b) sulfur- and nitrogen-containing species.

CONCLUSIONS

The real-time, comprehensive analysis provided by SIFT-MS makes it ideally suited for the rapid detection and quantitation of volatile metabolites which aids in the early diagnosis of Urinary Tract Infections. Also, having a unique set of volatile metabolites for each microorganism further aids the detection and identification of microorganisms associated with different types of UTIs. Early identification also helps in providing the right treatment to the patient.

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Microbiology

PA-96

Isolation from soil and development of culture methods for *Balamuthia mandrillaris*, a meningoencephalitis-causing

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1) Division of Bioscience and Laboratory Medicine, Hirosaki University Graduate School of Health Sciences, Japan
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Background

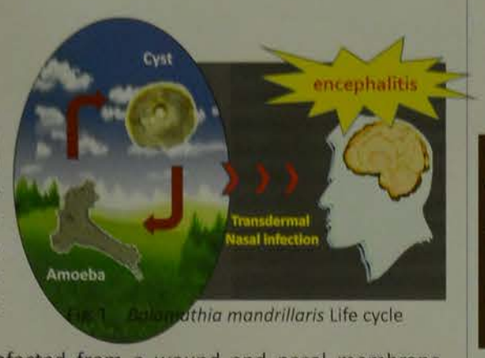


Fig.1 *Balamuthia mandrillaris* Life cycle

B. mandrillaris is classified as a free-living amoeba, living in a natural environment such as soil and water. This amoeba has two forms: trophozoite form and cyst form, and when it is exposed to adverse conditions such as dry, it changes to cyst form, enabling it to continue to survive for a long period of time. This amoeba is infected from a wound and nasal membrane, causing meningoencephalitis in mammals including humans.

Domestic death reports by amebic meningoencephalitis have been reported 10 cases until 2014. 8 cases among them are caused by *B. mandrillaris*, and many patients of the infected people had an underlying disease such as diabetes, hepatitis C, Sjogren's syndrome or the like. Therefore, although amoebic meningoencephalitis is believed to be an opportunistic infection caused by the decrease in immunity, since even infected people without any underlying disease have been also reported in Japan and abroad, it is an infectious disease that must be carefully monitored in the future.

Table 1 The occurrence of free-living Amoeba infection in people, Japan

Case number	Site	age/sex	year	source	history, Notices
1	Tokyo pref.	27/F	1976	*Acanthamoeba* spp.	Trip to the mountains
2	Yamagata pref.	56/M	1986	*B. mandrillaris*	Diabetes, Cellulitis of left upper limb
3	Okayama pref.	78/F	1989	*B. mandrillaris*	Mucocutaneous-ocular syndrome
4	Miyazaki pref.	72/F	1995	*B. mandrillaris*	HTLV-1 carriers
5	Nigata pref.	53/M	1995	*B. mandrillaris*	Stomach, nephrectomy due to stomach cancer
6	Saga pref.	25/F	1996	*N. fowleri*	No travel history,No contact with water
7	Miyazaki pref.	51/F	2006	*B. mandrillaris*	Epilepsy
8	Aichi pref.	56/F	2010	*B. mandrillaris*	No history
9	Tokusima pref.	68/M	2012	*B. mandrillaris*	Hepatitis C
10	Kyoto pref.	81/M	2014	*B. mandrillaris*	Sjogren's syndrome

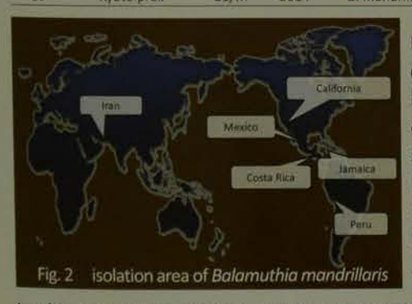


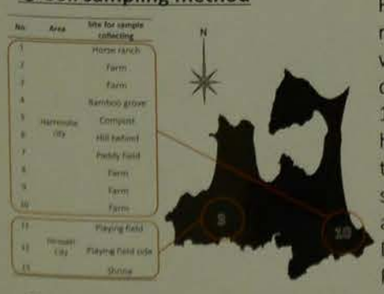
Fig. 2 isolation area of *Balamuthia mandrillaris*

In recent years, as an attempt to clarify the infection routes and habitats of this amoeba, flower pots of California, garbage of Iran, and soils of Peru and Mexican soil have been tested; in the most recent report, separation reports are made from a mud bath and the like of Jamaica.

In this study, we carried out the separation of *B. mandrillaris* from the soils of Aomori prefecture, a cold district in northern Japan. In addition, we attempted to identify the symbiotic bacteria of the separated *B. mandrillaris*.

Materials and Methods

○ Soil sampling method



For the soil samples, surface soil after removing fallen leaves on the surface were collected at a depth of about 10 centimeters. For the sample collections, 10 samples were collected at a general household in Hachinohe, 2 samples in the Hirosaki University campus, 13 samples in Hirosaki. The collected soils are cultured in addition to extracting DNAs to use for the *Balamuthia* specific PCR.

○ Isolation of *B. mandrillaris* from the environment

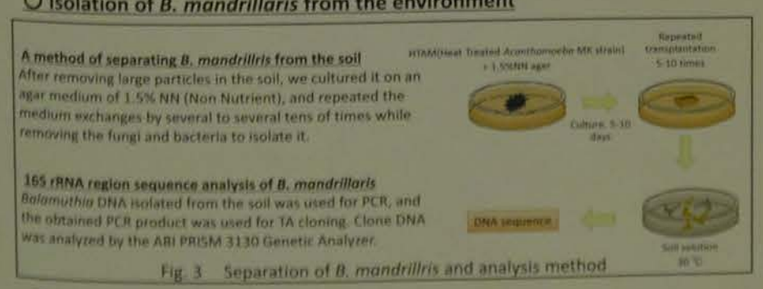


Fig. 3 Separation of *B. mandrillris* and analysis method

○ Environmental DNA extraction method

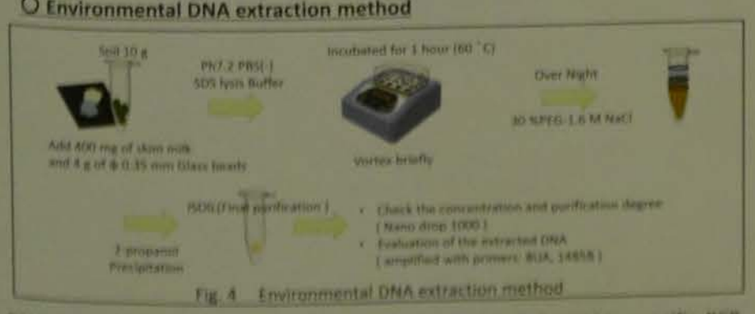


Fig. 4 Environmental DNA extraction method

DNA was collected from the soil, and amplified with *Balamuthia* specific PCR primer. For the DNA collection method, a three-step purification process by polyethylene glycol and isopropanol precipitation in addition to ISoil DNA extraction kit was employed for the purpose of removal PCR inhibitors (such as allophane and corrosion acid). For the DNA after purification, concentration and purification checks by nano drop are carried out to verify the judgment whether it can be used for PCR by actually performing PCR with the universal primer for the bacteria.

○ Existence proof of symbiotic bacteria

B. mandrillaris isolated from the environment is cultured for 2 weeks on a 1.5% NN agar to generate trophozoite form and cyst form in amoeba. Then, it was stained with SYTO9 and Propidium Iodide dye (PI) used in judging the viability of the cells, having affirmed the presence of symbiotic bacteria.

DNA of the isolated *B. mandrillaris* was extracted, and 16S rRNA was extended with the universal primer 1485B for bacteria, BUA to perform the clone analysis.

Results

○ *Balamuthia* specific PCR of soil DNA



Fig. 5 *B. mandrillaris* specific PCR of soil DNA

Non-specific reaction was seen in No.3 and 13, but a band was confirmed near 230 bp in soil No.1, 4, 7 and 11.

Amplification products confirmed in No.1 showed 99% homology with the already reported *B. mandrillaris* as a result of sequence analysis.

Table 2 No.1 : Homology comparison

No.	Accession No.	Strain	Query Cover	Ident
1	KT175741	*Balamuthia mandrillaris* strain V039 mitochondrion, complete genome	100 %	99 %
2	AF477017	*Balamuthia mandrillaris* isolate V433 small subunit ribosomal RNA gene	100 %	99 %
3	AF477015	*Balamuthia mandrillaris* isolate V416 small subunit ribosomal RNA	100 %	99 %
4	KT030673	*Balamuthia mandrillaris* strain SAM mitochondrion	100 %	99 %
5	KT030672	*Balamuthia mandrillaris* strain RP5 mitochondrion	100 %	99 %

○ Isolation of *B. mandrillaris* from No.1 soil

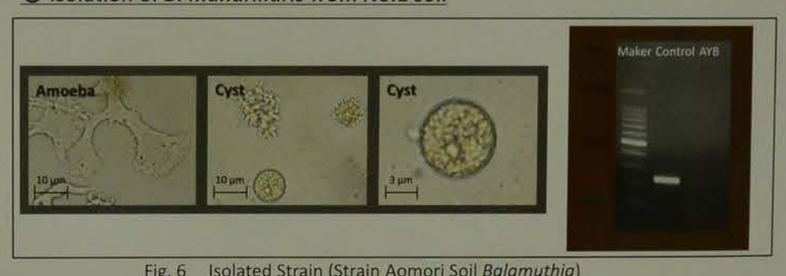


Fig. 6 Isolated Strain (Strain Aomori Soil *Balamuthia*)

Trophozoite form of amoeba was approximately 30 μm, cyst form was about 10 μm. As a result of PCR, a band was confirmed near 230 bp, and the genetic sequence of *B. mandrillaris* was also confirmed in the sequence analysis.

○ A phylogenetic tree of the neighbor-joining method

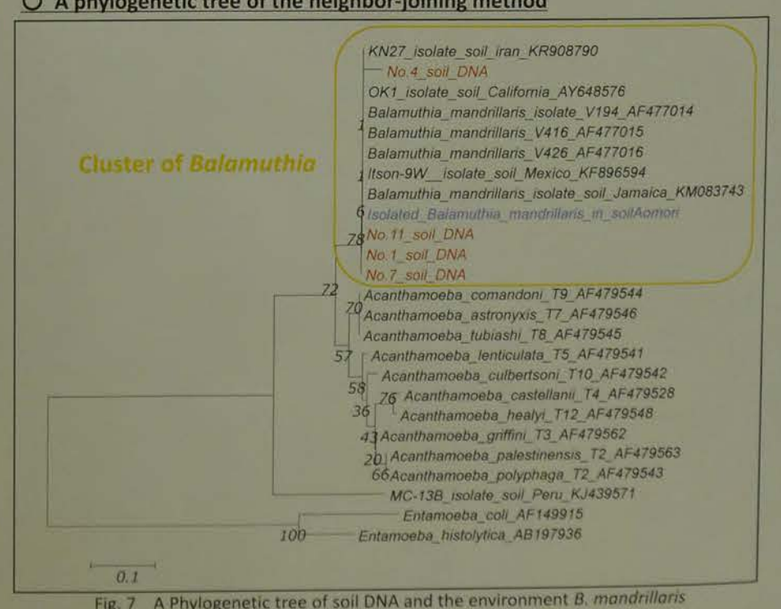


Fig. 7 A Phylogenetic tree of soil DNA and the environment *B. mandrillaris*

NJ tree was created by using the result of the sequence analysis. As a result, all the PCR samples were found to be classified into the cluster of *B. mandrillaris*.

○ Judging the viability of symbiotic bacteria by SYTO9 and PI



Fig. 8 Observation of the amoeba by confocal microscopy

AYB strains cultured for two weeks were stained with SYTO9 and PI. Bacteria incorporated in trophozoite form were dyed by SYTO 9, and found to survive in the amoeba body. Moreover, they also survive in the cyst form, suggesting that bacteria can survive for a long period of time in the amoeba body.

Table3 Sequence analysis of the symbiotic bacteria

Clone No.	Accession No.	Strain	Query Cover	Ident
1	KF943791	*Bradyrhizobium* sp.	100%	100%
2	GU294325	*Methylobacterium radiotolerans* strain	100%	99%
3	JF513347	*Pseudomonas aeruginosa*	97%	99%
4	GU294325	*Methylobacterium radiotolerans* strain	100%	99%

Conclusion

In 4 specimens among 13 specimens, a gene of *B. mandrillris* was detected. In addition, isolated *B. mandrillris* was obtained by culture under the environment of one specimen. This amoeba is seems to be able to survive under various environments regardless of weather conditions.

In the body of *B. mandrillris* separated from the soil, multiple bacteria live symbiotically, revealing that live bacteria have been captured as well in also the cyst of dormant period. It is suggested that bacteria and amoeba have a unique symbiotic system, and they are building a life circle while giving nutrients to each other.

In the future, we proceed to identify the symbiotic bacteria, aiming to build an amoeba culture system.

Microbiology

PA-97

Molecular typing of *Acinetobacter baumannii* clinical isolates from a hospital in Nonthaburi Province, Thailand

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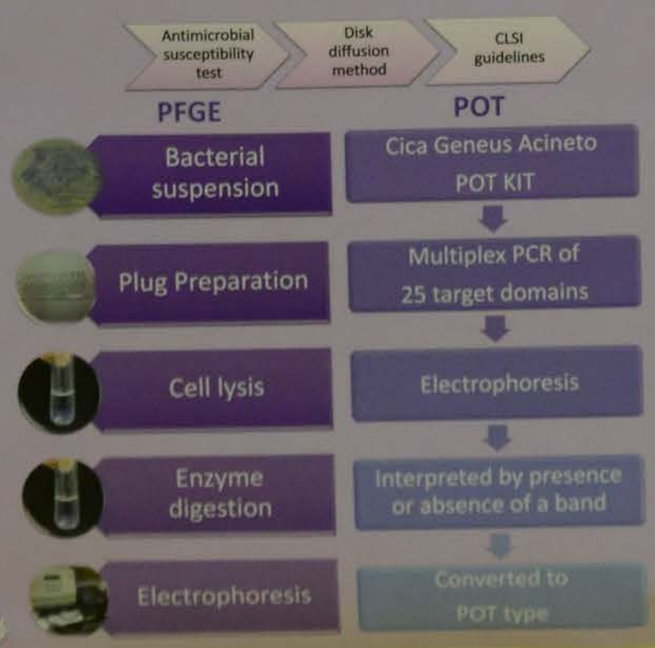
ABSTRACT

Acinetobacter baumannii is an opportunistic pathogen that becomes one of the most important nosocomial infections which are associated with elevated morbidity and mortality. Increasingly reports of multidrug-resistant (MDR) *A. baumannii* are shown worldwide. The aim of this study is to compare two molecular methods, pulsed-field gel electrophoresis (PFGE) and PCR-based open reading frame typing method (POT) for typing of *A. baumannii* clinical isolates. In this study, 62 non-duplicate isolates of *A. baumannii* were collected from 62 patients who admitted to a hospital in Nonthaburi, Thailand between 2012 and 2013. A total of 62 non-duplicate clinical isolates of *A. baumannii* were tested for antimicrobial susceptibility (the disk diffusion method) and performed molecular typing by PFGE and POT. All 62 isolates (100 %) were identified as MDR *A. baumannii*. Ten antibiotic drugs were tested for susceptibility, the lowest resistance rate was observed in only ampicillin/subactam (88.7 %). Eleven different PFGE types (PT) and 26 POT types were demonstrated. The predominant PT types was PT1 (59.7 %), followed by PT2 (9.7 %), PT3 (8.1 %), PT4 (6.5 %), PT5 (6.5 %) and 6 unique PT types. For POT method, type IX was the largest group (27.4 %), followed by type V (12.9 %), type VI (11.3%), type XV (4.8%). The discriminatory power using Simpson's Index of Diversity (SID) was higher in the POT (0.90, range 0.85-0.95) than PFGE (0.63, range 0.50-0.76). POT method may be useful for rapid genotyping of *A. baumannii* clinical isolates.

INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that becomes one of the most important nosocomial infections associated with elevated morbidity and mortality (1). Increasingly reports of multidrug-resistant (MDR) *A. baumannii* are shown worldwide. In Thailand, from 1998-2013, the susceptible rate of *A. baumannii* to carbapenem (imipenem) has decreased from 98.0 % to 33.1 % (2). Prevention and epidemiological surveillance of *A. baumannii* infections are important. Several of molecular typing or genotyping method should be necessary to study for understand of genetic relatedness (3). Pulsed field gel electrophoresis (PFGE) is the gold standard for epidemiological studies. However, PFGE is expensive and laborious (4). Another genotypic method of *A. baumannii* are available for commercial kit which developed by Suzuki M et al. (5) This method is PCR-based open reading frame typing method (POT) which are performed multiplex PCR of many specific genes of selected open reading frame (ORF) in small genomic islets and genomic islands of *A. baumannii* and then calculated the results in a binary manner to get POT type. This method can use conventional PCR machine and simple agarose gel electrophoresis set that have in many laboratory.

METHODS



RESULTS

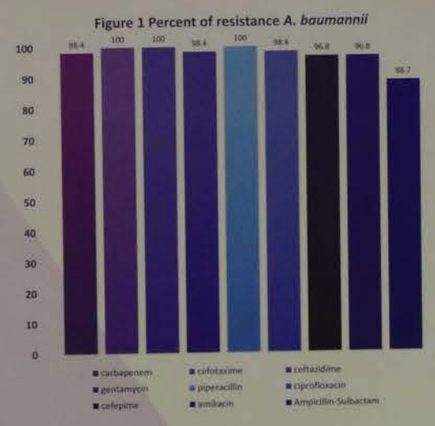


Table 1 Summary of dominant POT types

POT type	POT1- POT2- POT3	Number of isolates	PFGE pattern (number of isolates)
IX	122-53-43	17 (27.4 %)	PT1 (14), PT2 (2), PT7 (1)
V	122-53-42	8 (12.9 %)	PT1 (8)
VI	8-63-48	7 (11.3%)	PT5 (4), PT4 (3)
XV	122-21-43	3 (4.8%)	PT1 (3)
X	104-30-59	2 (3.2%)	PT3 (2)
I	104-63-26	2 (3.2%)	PT1 (1), PT6 (1)
VII	122-53-47	2 (3.2%)	PT1 (2)
III	122-58-59	2 (3.2%)	PT1 (1), PT2 (1)
IV	122-58-27	2 (3.2%)	PT1 (2)

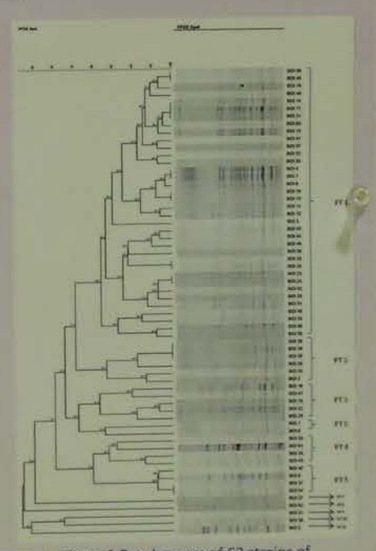


Figure 2 Dendrogram of 62 strains of *A. baumanni* generated by Bionumeric V.7.5

The Simpson's Index of Diversity (SID) with 95% confidence intervals for POT method (0.898, range 0.845-0.951) revealed high value. However, in PFGE method, SID was 0.628, range 0.495-0.761.

CONCLUSION

This is the first pilot study in Thailand for *A. baumannii* clinical isolates that comparison between PFGE and POT method. The discriminatory power using Simpson's Index of Diversity (SID) with 95% confidence intervals revealed that POT method had higher SID (0.90, range 0.85-0.95) than PFGE (0.63, range 0.50-0.76). POT method may be useful for rapid genotyping of *A. baumannii* clinical isolates.

ACKNOWLEDGEMENTS

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PA-98

Improvement of Group B streptococc screening positive rate for pregnant woman to reduce neonatal infection

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Introduction

Reproductive tract which 10-35% of healthy women can be found in B Streptococcus (Group B Streptococcus, GBS), although it is not the disease, but may causing newborn infections, sepsis, meningitis, nerve lesions, severe potentially lethal. United States Centers for Disease Control do advocate screening of B streptococci in 35 to 37 weeks of pregnancy, it can effectively prevent the risk of newborn infection. Therefore, Department of Health since 2012 years in April, health check screening program for pregnant women with insurance payments. So how to improve the positive rate of GBS screening, is a very important thing. In January 2013, Department of Laboratory Medicine reference peers hospital GBS quality indicator set at 15%, but from January to September there are three months less than the target value, to proceed with a project to improve the planning stage. In January 2014, Department of Laboratory Medicine in accordance with National Health Department suggestions, amendment GBS quality indicator set at 18%.After the implementation of the project to improve the program, GBS positive rate has improved significantly.

Methods

According to statistics, Taiwan's maternal GBS colonization rate of 18%, set in our laboratory quality indicators. 2012--2013 there were seven months less than the target of 18%, and the ups and downs changes, then the related improvement measures.

1.Change test kits:

(1)2014 by the LIM broth can be changed to produce pigment proliferation identification medium Strep B Carrot Broth ™. To increase the sensitivity and specificity of screening to reduce the incubation time and the use of supplies.



LIM broth



Carrot broth

(2)2015 GBS DetectTM substituted BAP agar, can be induced by γ-hemolytic GBS produce β-hemolytic. Simplified screening γ-hemolytic B streptococcus steps and add all group B streptococcus isolation rate.

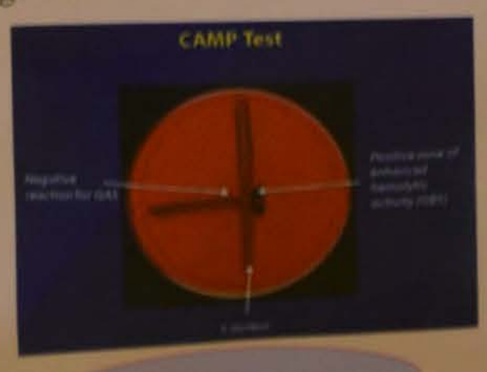


BAP agar



GBS detect™

2. Change the test method:



CAMP test

CAMP test confirmation test, to obtain a single colony for Drug Susceptibility Testing

3. Changing the specimen container :



Black tube containing "Charcoal" interpretation affects the color, change with the blue head tube.

4. Change preservation methods:
Not immediately inoculated specimen tube and stored at 4 ℃. Within 24 hours of specimen inoculation, culture positive rate can be increased.

5. Different specimen collection unit, GBS positive rate detection rate statistical analysis:
Outpatient departments specimen collected by the attending physician, GBS-positive rate to maintain a consistent. Replace the delivery room because residency, the positive rate dropped by 10%. The statistics provide maternity room, the importance of advocacy correct specimen collection.

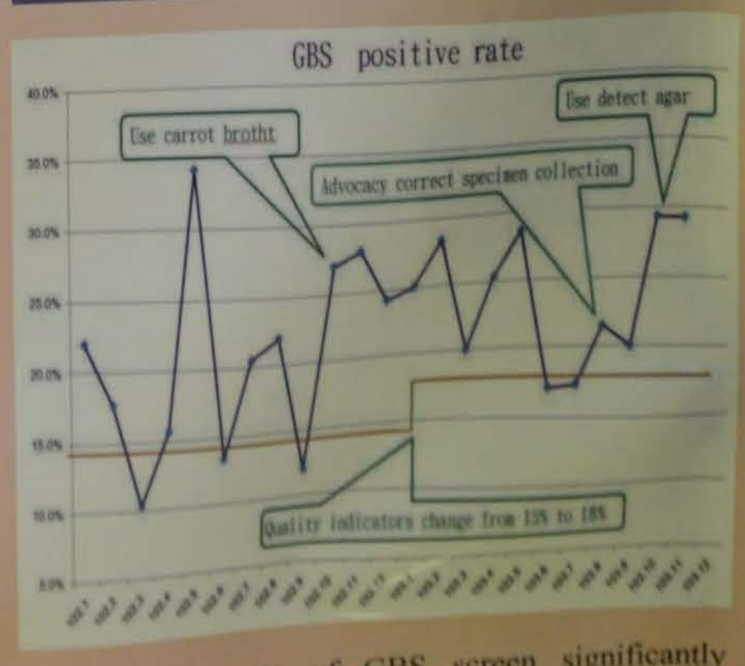
	Outpatient department			Maternity department		
2014	positive case	total	positive rate	positive case	total	positive rate
January	15	66	23 %	8	26	31 %
February	21	76	28 %	5	16	31 %
March	13	69	19 %	5	21	24 %
April	17	67	25 %	5	20	25 %
May	23	72	32 %	3	19	16 %
June	10	53	19 %	2	17	12 %
July	16	90	18 %	3	20	15 %

6. Specimen collection methods standardization:



7. Continuous monitor of GBS positive rate monthly.

Results



The positive rate of GBS screen significantly increased and remained 25~28% since October 2013, after the implementation of the improvement project.

Conclusion

For pregnant women Streptococci screening, prevention strategies should be prenatal (35-37 weeks gestation) comprehensive screening. State Health Department recommends the correct result more important than timeliness. GBS test positive, should be treated with antibiotics. Prevention of neonatal infection regret and reduce long-term health care costs.

Microbiology

PA-99

Antibacterial and antibiofilm activities of *Scrophularia ningpoensis* hemsl extracts against *Klebsiella pneumoniae*

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Background

Scrophularia ningpoensis hemsl has been traditionally used in Vietman for treatment of antibacteria, atopy, pimple, tonsillitis, angina, encephalitis for a long time. The main objectives of this study were to evaluate the antibacterial activity of the *Scrophularia ningpoensis* hemsl extract on planktonic growth and biofilm formation of *Klebsiella pneumoniae*.

Method

Antibacterial activity was conducted using disc diffusion assay and minimum inhibitory concentration (MIC) / minimum bactericidal concentration (MBC) were determined using the broth micro dilution method in accordance to Clinical and Laboratory Standards Institute guidelines(CLSI).
Furthermore, cytotoxicity on L929 were assessed using Animal cell culture for the proliferation test(MTT cell assay) and the biofilm forming capacity of the *K. pneumoniae* were determined using the colony forming unit (CFU) assay.

Results

The extract exhibited considerable antibacterial activity. *K. pneumoniae* was susceptible to the extract with the MIC and MBC of 0.1875 and 1.5 ㎎/㎖ respectively. Cytoxicity test in L929 showed no sign of toxicity at the concentration of 0.75㎎/㎖ and at the same concentration the extract caused inhibition of bacterial biofilm formation.

Conclusion

The extract of *Scrophularia ningpoensis* hemsl possesses an in vitro antibacterial antibiofilm activities against *K. pneumoniae*, with no sign of cytoxicity on L929.

•Keyword: Anitbacterials, Biofilm, *Klebsiella pneumoniae*, *Scrophularia ningpoensis* hemsl

Table 1. Information on the plants used and report on evidence of their activities

Botanical Name	Traditional uses
Scrophularia ningpoensis Hemsl	Antibacteria, Atopy, Pimple, tonsillitis, Angina, Encephalitis, Hypertensive petechial fever, scrofula
Buddleja officinalis Maxim	Rheumatism
Clinopodium chinense (Benth.) Kuntze	Gonorrhea, Hematuria, Epistaxis

Table 2. Antibacterial activity of the methanol extract of *Scrophularia ningpoensis* Hemsl against *K.pneumoniae* (n=3)

Antibacterial activity	*Scrophularia ningpoensis* Hemsl
MIC	0.1875mg/ml
MBC	1.5mg/ml

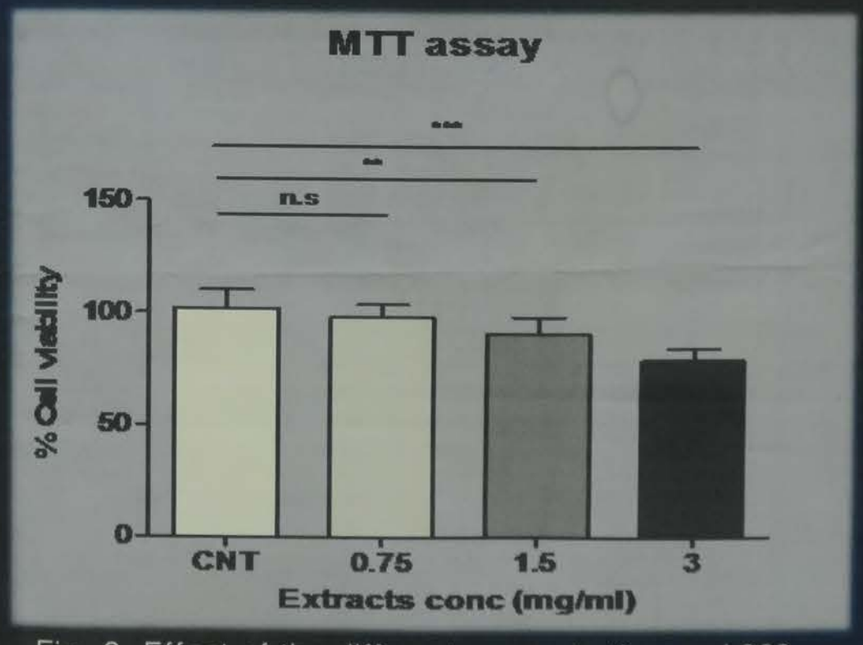


Fig. 2. Effect of the different concentration on L929 cells. (**p< 0. 0 5, ***p<0.001)

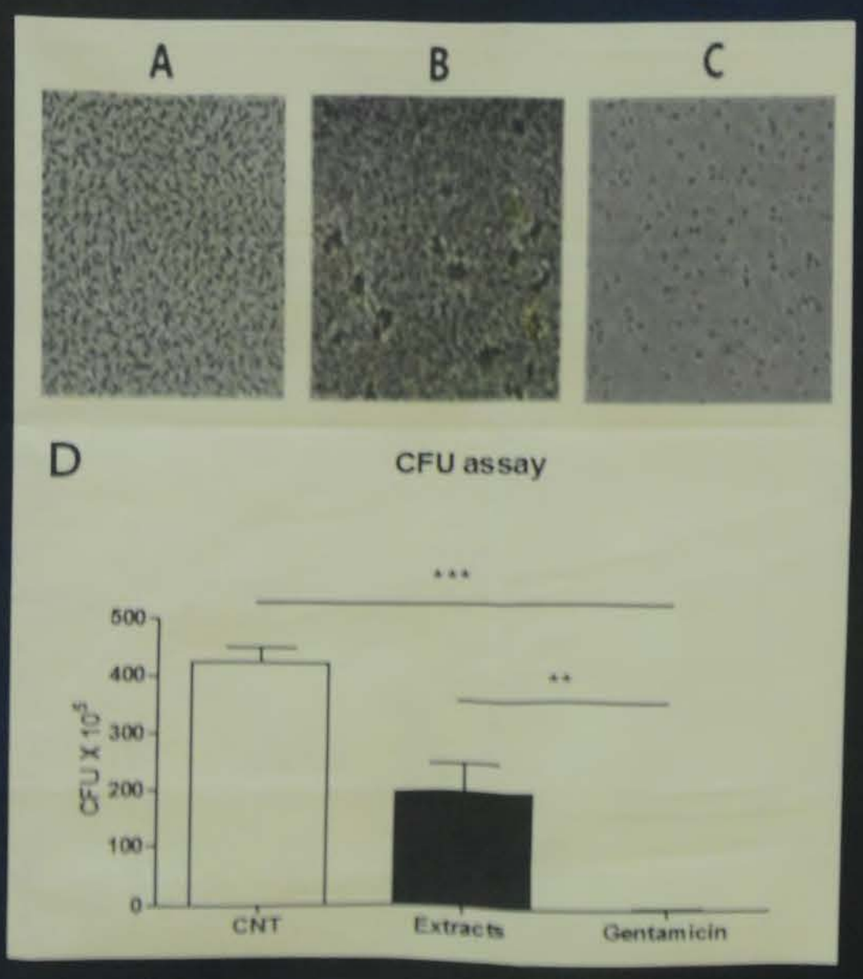


Fig. 3. Microscopic images (X400) on biofilm formation of *K. pneumoniae* treated with *Scrophularia ningpoensis* Hemsl extract (**p= < 0. 0 5, ***p<0.001) A:CNT(Negative control), B:*Scrophularia ningpoensis* hemsl extract C: gentamicin D:Colony forming unit (CFU) assay(CNT : only *K.pneumoniae*, Extracts : *Scrophularia ningpoensis* hemsl extract (0.75mg/m)l, gentamicin (200ug/ml)).

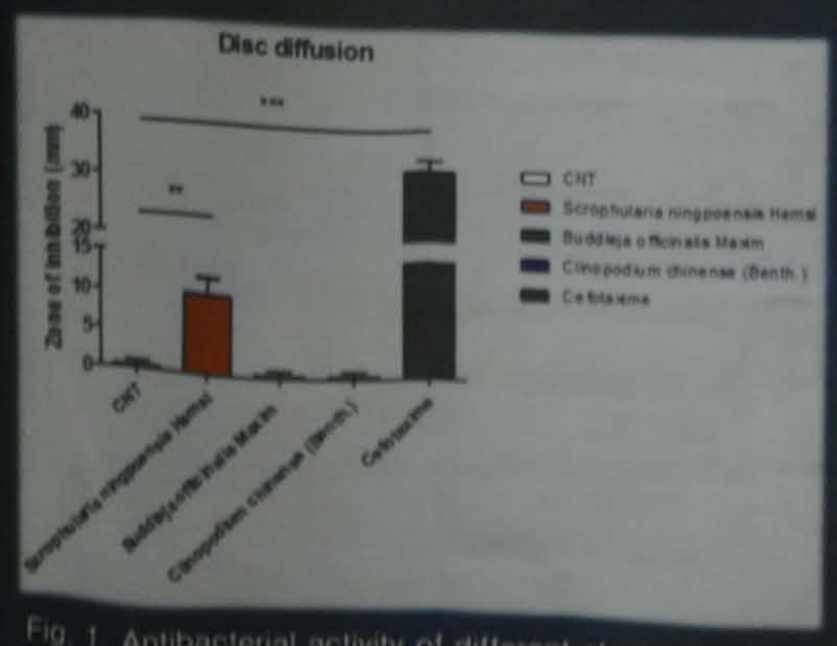


Fig. 1. Antibacterial activity of different plant extracts (**p< 0. 0 5, ***p<0.001)

HiT 대전보건대학교