CASE REPORT

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The genus *Dyella* spp. bacteremia from hemodialysis blood culture: a case report

Monchai Siribamrungwong[1], Kwanchon Jearakitiwanich[2*]

[1] Department of Internal Medicine, Lerdsin General Hospital, College of Medicine, Rangsit University, Bangkok, Thailand.
[2] Department of Clinical Pathology and Medical Technology, Lerdsin General Hospital, Bangkok, Thailand.

KEYWORDS
Dyella spp.;
Automated blood culture;
Matrix- assisted laser desorption/ ionization;
Mass spectrometry.

ABSTRACT
This study presents a patient identified to have *Dyella* spp., a rare genus of bacteria, bacteremia. *Dyella* spp. cannot be identified through biochemical testing methods. At present, Matrix-assisted laser desorption/ionization is applied to differentiate the types of microbes. However, there is a limitation in distinguishing the specific type of bacteria because of the limitation of library databases. The 16S rRNA sequencing is crucial for differentiating the rare genus of bacteria that are improbable to recognize by mass spectrometry technique. This report highlights the discovery of *Dyella* spp. in patient blood culture, emphasizing the challenges in identification by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS), an ordinary technique. The instruction provided in this report aims to improve the application of this high-technology machine to accurately differentiate the rare genus of bacteria, ultimately enhancing patient care and treatment outcomes.

***Corresponding author:** Kwanchon Jearakitiwanich, MT, MSc.Department of Clinical Pathology and Medical Technology, Lerdsin General Hospital, Bangkok, Thailand. Email address: kwanchon2529@gmail.com
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Introduction

Dyella spp. are aerobic gram-negative rods, small, yellow, classified under gamma- proteobacteria. In 2005, a novel finding related to the genus *Dyella*, in the family *Xanthomonadaceae*, was reported, with the species *Dyella japonica* mentioned in a previous report[1]. The genus *Dyella* includes six species: *D. japonica*, *D. koreensis*, *D. ginsengisoli*, *D. marensis*, *D. soli* and *D. terrae*[2]. This genus is typically isolated from water, soil, and other environment sources[2, 3]. All *Dyella* spp. are environmental isolates and have not reported to cause human infections. Human infections caused by *Dyella* spp. are extremely rare, and their pathogenicity in humans remains unclear. In Thailand, the first case of infection by *Dyella japonica* in hemodialysis patients was reported. Contaminated hemodialysis may have caused bacteremia. The severity of bacteremia was mild, and the patient responded well to antibiotic therapy[1]. These environmental bacteria are commonly found in hemodialysis patients, who are at high risk for opportunistic infections[1].

The methods for identification of this genus are difficult and challenging for clinical microbiology laboratories. Conventional and automated biochemical identification methods failed to identify these bacteria. The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) also misidentified these bacteria due to certain limitations of the technique[4].The spectrum of the *Dyella* spp. in database for MALDI-TOF MS identification includes limited isolates. To further confirm the identification may use 16s rRNA gene sequencing. After confirming by 16s rRNA then generates a reference spectrum in the MALDI-TOF MS database for identification of bacteria next patients.

Herein, we report a patient identified to have *Dyella* spp., a rare genus of bacteria, which cannot be identified through biochemical testing and MALDI-TOF MS.

This report demonstrates the usefulness of molecular methods for identifying uncommon bacteria from clinical specimens.

Case history

A 35-year-old woman presented to the emergency room with fever and chill symptoms for one day during a hemodialysis session. Her underlying diseases were end-stage renal disease (ESRD), diabetes, hypertension, and secondary hyperparathyroidism. The physician sent three blood culture samples drawn from different sites: the A-line, the V-line of the dialysis permanent catheter, and the peripheral line. The samples were placed in BACTEC Plus Aerobic/F bottles and incubated in a BACTEC FX automated blood culture system (Becton Dickinson, USA). All bottles showed positive results after 48 hours of incubation (Table 1).

Table 1 Time to positive for all blood cultures from hemodialysis patient

Blood collection position	Time to positive
A-line (dialysis permanent catheter)	17 hours and 33 minutes
V-line (dialysis permanent catheter)	19 hours and 33 minutes
Peripheral line no.1	3 days, 13 hours, and 38 minutes
Peripheral line no.2	No growth after 3 days
Peripheral line no.3	1 day, 14 hours, and 34 minutes
Peripheral line no.4	1 day, 11 hours, and 34 minutes

Materials and methods

Ethical approval

In this study, *Dyella* spp. isolate was cultured from left-over blood bottle after a routine diagnostic examination. The isolate was then anonymized with no patient's data links to protect patients. This case report was approved by the Ethics Committee for Human Research, Lerdsin Hospital, Department of Medical Services (Certificate of approval number LH671044).

Sample processing

The specimens from the positive bottles were cultured on solid media, including 5% sheep blood agar, MacConkey agar, and chocolate agar as the routine of clinical microbiologic laboratory protocol. The morphology of the bacteria was demonstrated as Gram-negative bacilli (Figure 1). Specific colony characteristics on blood agar were light yellow color and less than one mm. in size (Figure 2). Specimens from the plates consistently demonstrated negative bacilli on Gram staining with a positive oxidase test. Identification by mass spectrometry with the MALDI-TOF MS Sirius (Bruker Daltonics, Germany)[4] could not determine the species, yielding an identification score of < 1.4, which is considered "unreliable" according to the manufacturer's guidelines.

The culture plates were incubated for 16 hours and then retested for species identification and antimicrobial susceptibility for Gram-negative bacilli. The species identification results remained the same, with an identification score of <1.4[4]. Antimicrobial susceptibility testing using the dilution method was performed, with results interpreted as minimum inhibitory concentration (MIC). However, interpreting the MIC results as susceptible, intermediate, or resistant according to The Clinical & Laboratory Standards Institute (CLSI) guidelines was challenging if precise genus and species identification were undetermined[5].

Result

To further identify the species, the laboratory sent the sample to another laboratory for automated biochemical testing with a ready-to-use test kit (Sensititre, United Kingdom). The preliminary result was *Elizabethkingia meningoseptica*, which was reported along with the antimicrobial susceptibility result. Subsequently, the laboratory conducted the additional analysis using another automated biochemical test (Phoenix, USA) and a different MALDI-TOF MS Sirius machine. The automated biochemical test (Phoenix) identified the bacterium as *Spingomonas paucimobilis*, whereas the MALDI-TOF MS Sirius machine insistently unsuccessful to identify the species. According to the manufacturer's and CLSI recommendations[5], an extraction method was used to improve sample preparation, increasing the likelihood of detecting bacterial proteins and obtaining a better spectrum from the MALDI-TOF MS. This preparation directed the identification scores of 1.4-1.5 for *Dyella jiangningensis*, but the score was still categorized under "No reliable identification."

The analyses were inconclusive, so molecular biology techniques were essential to advance the identification. Specifically, 16S rRNA sequencing, defining the bacterial base sequence, was applied[1, 6]. The sequence base of the sample was compared to the reference strains in the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sample's base sequence needed a percent identification score of at least 98.5%, similar to the database's standard strains[6]. The comparison revealed that the patient's sample had percent identification scores of 99.03% and 99.02% for *Dyella* spp. and *Dyella jiangningensis*, respectively. Therefore, it was preliminarily concluded that the patient's sample was the *Dyella* spp.

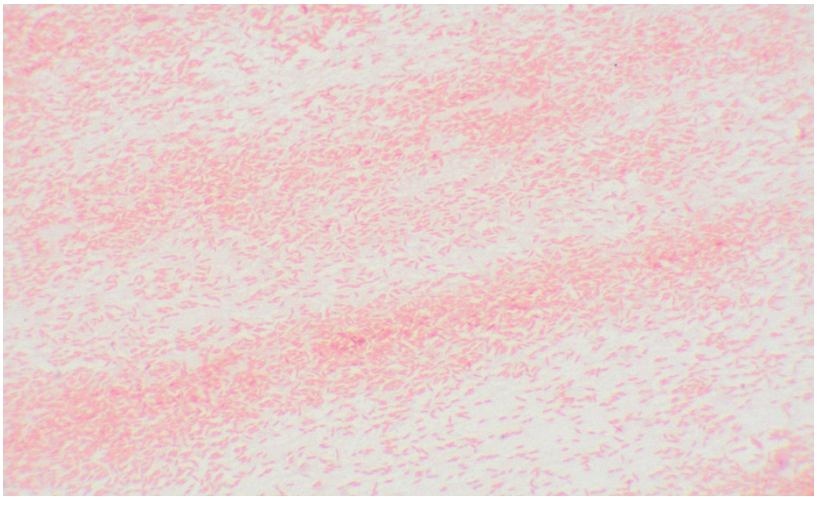

Figure 1 The Gram stain morphology of bacteria from blood culture.

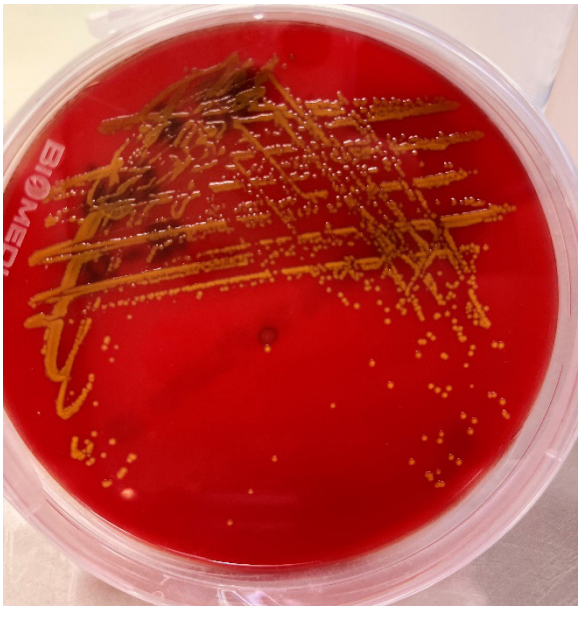

Figure 2 The morphology of bacteria on blood agar plate.

Discussion

The genus *Dyella* spp. was firstly discovered in 2005 in Japan from environmental samples. It was found to be closely related to bacteria in the family *Xanthomonadaceae*. Reports of *Dyella* spp. from clinical specimens have been limited[7,8]. In 2007, Kiratisin P. reported *Dyella japonica* bacteremia in a hemodialysis patient, the first report of *Dyella* spp. human infection[1]. Duus LM reported *Dyella* spp. colonization in sputum from patients with cystic fibrosis[7, 8]. In this study, the bacterium isolated from blood specimens is *Dyella* spp., genetically related to *Dyella japonica*[9].

Conventional and commercial biochemical tests used for routine species identification have proven inaccurate for *Dyella* spp. Previous tests have often misidentified it as *Elizabethkingia meningoseptica*, or *Stenotrophomonas maltophilia*[7, 10].

These discrepancies could be due to variations in the types of culture media used by different manufacturers, leading to inaccurate species identification. In this study, two different commercial biochemical tests produced conflicting results. The MALDI-TOF MS method also reported "No reliable identification," even with additional sample preparation using extraction methods. The lack of reliable identification reports may be because *Dyella* spp. is rare, and the database for the MALDI-TOF MS has few reference strains, resulting in the scores below 1.7[7, 10].

Once routine methods are unsuccessful in identifying the bacterium accurately, more precise methods like 16S rRNA sequencing are necessary. This study used 16S rRNA sequencing, which showed that the sample's sequence was more than 98.5% like the three strains in the NCBI database. Since *Dyella* spp. is uncommon, few researchers studied its sequences, and only some sequences are available in the NCBI database. Consequently, this study could only preliminarily identify the sample as *Dyella* spp. A reference spectrum for this bacterium was created in the MALDI-TOF MS system to aid future species identification[7, 8].

Conclusion

The antibiotic piperacillin-tazobactam was a treatment for this case. The genus *Dyella* spp. is mostly isolated from environmental sources such as soil or air, but there have been increasing reports of its presence in clinical specimens, particularly in the patients at risk for opportunistic infections. Laboratory identification tests have difficulty accurately identifying *Dyella* spp. Conventional biochemical tests cannot distinguish the strain, and even using MALDI-TOF MS has limitations due to the limited reference strains in the database, resulting in unreliable identifications. The authors recommend that once biochemical tests or MALDI-TOF MS cannot identify the bacterium or raise doubts about its identification, it is necessary to confirm the species using 16S rRNA sequencing. The recommendation should be followed for all cases, and the data should be reported to improve future species identification capabilities.

Take home messages

This environmental bacteria genus *Dyella* spp. is a rare case of human infection. However, bacteria can cause bacteremia in patients who are at risk of opportunistic infection.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Monchai Siribamrungwong: Conceptualization, Methodology, Writing – Review & Editing (clinical perspective).

Kwanchon Jearakitiwanich: Methodology, Formal analysis, Writing Original draft, Writing – Review & Editing (laboratory perspective).

Data availability

Data available on request due to privacy/ethical restrictions

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