A CASE OF *NECROPSOBACTER ROSORUM* BACTERAEMIA

Pirathaba KJ[1], Ishak N[1], Ngoo SKMH[2], Hashim R[2], and Samsudin N[2].
[1]Microbiology Unit, Pathology Department, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur, Malaysia
[2]National Institute of Health, Persiaran Setia Murni, Setia Alam, 40170 Shah Alam, Selangor

Correspondence:
Karshini Jeya Pirathaba,
Microbiology Unit, Pathology Department,
Hospital Kuala Lumpur,Jalan Pahang,
50586 Kuala Lumpur, Malaysia
Email: karshinijeya28@gmail.com

Abstract

Necropsobacter rosorum is a gram-negative organism previously classified under the family *Pasteurellaceae.* It has been isolated from the gastrointestinal and respiratory tracts of mammals. However, there is very little information available about how *N. rosorum* infects humans. Due to a paucity of information in the database, commercial identification methods such as the VITEK identification system or the Analytical Profile Index (API) frequently misidentify this organism. Thus, it is a challenge for laboratories to accurately identify *N. rosorum*. By using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF), we were able to identify *N. rosorum* in a patient with sepsis secondary to wet gangrene of the left big toe. This case report aims to shed light on the application of advance diagnostic tests for the early and accurate detection of rare human pathogens. It also contributes to a better understanding of the diseases associated with this bacterium.

Keywords*:* Bacteraemia, *Pasteurellaceae*, *Necropsobacter rosorum*, MALDI-TOF, Intraabdominal Abscess

Introduction

Necropsobacter rosorum is a facultatively anaerobic, gram-negative, pleomorphic rod or coccoid bacteria that is nonmotile, non-haemolytic, and nonpigmented. It was first identified by Mannheim and his colleagues from the lung tissues of a guinea pig (1). Initially, this bacterium was classified in the family *Pasteurellaceae.* However, Christensen et al. reclassified this bacterium as *Necropsobacter* gen nov with a single species, *N. rosorum* in 2011 (2). In 2015, *N. massiliensis* was included under this genus (3). *N. rosorum* has previously been isolated from the respiratory and gastrointestinal tracts of mammals (1, 2, 4). However, there are extremely few cases of infections in humans. We present a case of bacteremia secondary to wet gangrene of the left big toe caused by *N. rosorum. N. rosorum* was identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) Microflex LT and confirmed with 16S rRNA gene sequencing.

Case report

A 67-year-old man with type 2 diabetes presented to the emergency department with a 6-day fever and swelling in his left foot. The left foot was painful, with bluish-black discoloration and foul-smelling discharge over the left big toe. On admission, he was in sepsis with evidence of pyrexia (39.4°C), leucocytosis of 21.0 x 10^3 cells/µL: predominantly neutrophils (reference range: 4.00-10.00 x 10^9/L), and a raised C-reactive protein level of 49.9 mg/L (< 5 mg/L). His random blood sugar was 8 mmol/L (reference range: 3-11.1 mmol/L). He received piperacillin-tazobactam as empirical therapy. Radiological findings showed evidence of chronic osteomyelitis over the left big toe. He then underwent ray amputation of his left big toe. The operative findings were wet gangrene of the left big toe, extending to the first metatarsal with exposed tendons, and pus discharge.

Blood cultures were performed on the day of admission using BD Bactec Plus Aerobic/F and BD Bactec Lytic/10 Anaerobic/F vials, which were incubated using BD BACTEC™ FX. The aerobic bottle blood culture was positive after 24 hours of incubation. The initial gram stain showed gram-positive cocci in chains and gram-negative bacilli. The sample was plated onto 5% Sheep Blood Agar Plate (BAP) and MacConkey Agar (MAC) and incubated at 35°C in ambient air.

Two colonies grew, with the gram-positive cocci in chains identified as *Streptococcus oralis,* susceptible to penicillin. The other organism appeared as a large, greyish, convex, non-haemolytic, non-pigmented colony on BAP (Figure 1) and a flat, non-lactose fermenting colony on MAC. The colony's gram stain revealed gram-negative coccobacilli bacteria (Figure 2). The isolate tested oxidase and catalase positive. It produced acid butt and slant, with gas production but no hydrogen sulphide, on Triple-sugar iron agar, and was capable of fermenting glucose on Hugh and Leifson's oxidative fermentation medium.

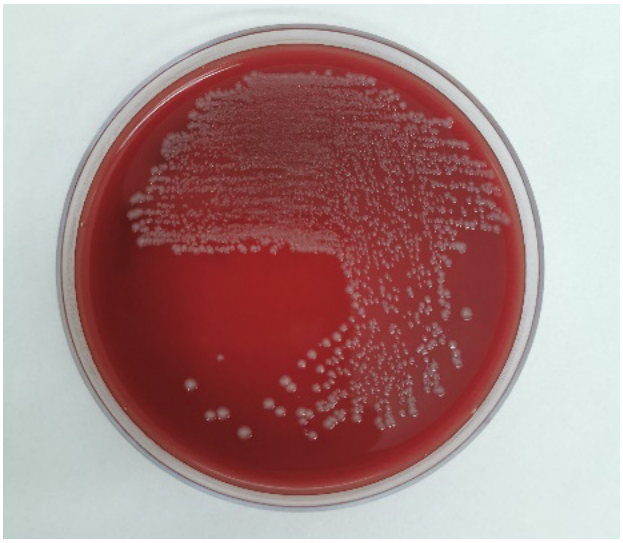

Figure 1: Isolate on BAP

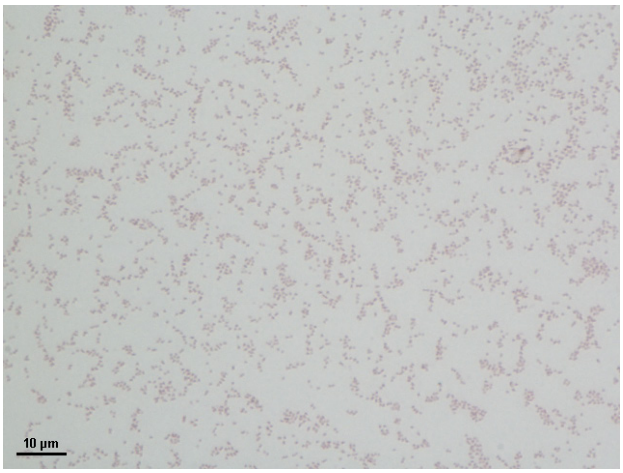

Figure 2: Gram stain from BAP colony

Biochemical testing showed a positive reaction for methyl red and arginine decarboxylase, while indicating negative reactions for indole, citrate, urea, ornithine decarboxylase, lysine decarboxylase, and motility. Analytical Profile Index (API) 20E and VITEK® 2 GN (bioMérieux, France) were unable to achieve any conclusive results. The isolate was identified as *N. rosorum* via matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF), Bruker Microflex LT with a score of 2.36.

The isolate was sent to the national reference laboratory for genotypic confirmation where partial 16S rRNA gene amplification was performed. Bacterial DNA was extracted using Maxwell® 16 Blood DNA Purification Kit by Maxwell® 16 MDx (Promega) according to the manufacturer's protocol. The universal amplified ribosomal region (UARR) was amplified using the following primers: U1F: 5'-CTYAAAKRAATTGRCGGRRRSSC-3', U1R: 5'-CGGGCGGTGTGTRCAARRSSC-3' (Integrated DNA Technologies, IDT, Coralville, Iowa, United States) in 25 µL reaction volumes containing 3 µL of extracted DNA template, 12.5 µL of MyTaq™ HS DNA Polymerase (Meridian Bioscience Inc., Ohio, United States), 1 µL of 20 µM of each primer and 7.5 µL of sterile molecular grade water. The amplification was performed in a Mastercycler® Nexus GX2 (Eppendorf®) under the following protocol: one cycle of pre-denaturation at 95°C for 60 sec followed by 25 cycles of denaturation at 95°C for 15 sec, annealing step at 63°C for 30 sec and an extension step at 72°C for 15 sec. The PCR products were separated on a 1.5% agarose gel via electrophoresis and sent for sequencing. The DNA sequences of the partial 16S gene were analysed with BioEdit version 7.2.5 and compared with 16S rRNA sequences deposited in the GenBank database. The organism was identified as *N. rosorum* (100% query cover and 98.99% identification).

Antibiotic susceptibility testing was performed by the gradient diffusion method using E-test according to Clinical and Laboratory Standards Institute (CLSI M45, 2015) (5). Susceptibility breakpoints for *Pasteurella* spp were used for interpretation. Isolates were susceptible to amoxycillin-clavulanate, ceftriaxone, tetracycline and trimethoprim-sulfamethoxazole, while resistant to erythromycin (MIC 32 mcg/mL) and penicillin (MIC 2 mcg/mL) (Table 1). Bone specimen culture was negative.

Table 1: Minimum inhibitory concentration of antimicrobial against the *Necropsobacter rosorum* isolate

Antimicrobial	Minimum inhibitory concentration (MIC) (mcg/mL)
Amoxycillin/clavulanate	0.38
Erythromycin	32
Ceftriaxone	< 0.016
Penicillin	2
Tetracycline	1.0
Trimethoprim-sulfamethoxazole	0.094

Intravenous piperacillin – tazobactam was continued for four days as the patient showed significant clinical improvement. He was discharged well with oral amoxycillin-clavulanate 625 mg three-time daily (TDS) for two weeks. The wound was clean prior to discharge.

Discussion

There is little information available about *N. rosorum* causing human infections. Tan et al. describe five patients with *N. rosorum* bacteremia in a case series. It was associated with either intraabdominal infection or bone and soft tissue infection in the pelvic region (6). A similar case of intraabdominal infection was reported in a 55-year-old gentleman with perforated gastric carcinoma (7). Most patients were immunocompetent, with no underlying medical illness.

Our patient had no signs or symptoms of an intraabdominal infection, and his biochemical markers for liver function were within the normal range. The only source of infection identified was his infected left big toe. According to Aroa et al, only 69% of diabetic foot patients have positive bone cultures (8). The possibility of a culture-negative bone specimen could be either due to prior antibiotic therapy given before specimen collection or because the specimens sent for culture were taken from the uninfected bone site.

It is unclear if *N. rosorum* infection has any associations with animal contact that are seen in other *Pasteurellaceae* species. The prognosis of this infection is still unknown. Only one patient succumbed to death in the case series of Tan et al. (6), and it is unclear if *N. rosorum* was the possible cause of death as the patient had a polymicrobial infection. Our patient was discharged well.

Our isolate shows similar phenotypic characteristics as described by Christensen et al. and Tan et al. (1, 6). The isolate is oxidase and catalase positive and ferments glucose on oxidative fermentative (OF) medium. The phenotypic characteristics of *N. rosorum* are similar to those of *Pasteurella* spp. of clinical importance. It can be misidentified when using conventional identification systems such as the VITEK 2 identification system or the Analytical Profile Index (API) (6, 7). Simple biochemical tests aid in differentiating these two organisms. *Pasteurella* spp does not grow on MacConkey agar and it produces indole.

MALDI-TOF has been a promising tool that allows us to recognise *N. rosorum* as a human pathogen. Since *N. rosorum* is already included in the library database, it is reliable, cost-effective, and easy to perform compared to 16S rRNA gene sequencing. Since this was our first encounter with *N. rosorum* we performed gene sequencing to validate the identification.

The selection of antibiotic breakpoints was challenging, as no information was found in CLSI or EUCAST. CLSI breakpoints for non-enterobacteriaceae were used by both Tan et al. (6) and Jin et al. (7) to interpret antibiotic susceptibility. The antibiotic susceptibility in this present case was determined according to the interpretative breakpoints for *Pasteurella* spp. (CLSI M45, 2015) as guidance, with the reasoning that *N. rosorum* previously belonged in the family *Pasteurellaceae.* Thus, it is important to accurately identify and test for antibiotic susceptibility to determine the appropriate treatment option for patients.

Conclusion

Apart from 16S rRNA sequencing, MALDI-TOF is an option to accurately identify *N. rosorum*. At present, there are still gaps in information regarding its epidemiology, association with animal contact, virulence factors, antibiotic susceptibility and resistance, and mortality and morbidity. The importance of *N. rosorum* as a human pathogen requires further study.

Acknowledgement

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Conflict of interest

The authors declare that they have no competing interests.

Financial support

There is no financial support received for the study.

Ethics approval

This research was registered in the National Medical Research Registry (NMRR) Research NMRR ID-22-02930-5EV. The Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH) has exempted this study from ethical approval as this study is a case report.

Informed consent

Verbal consent was obtained from patient before the case report. Written consent was not obtained because the data was collected retrospectively, the subject was not physically presence at the healthcare facility.

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