

Ralstonia mannitolilytica: an emerging multidrug-resistant opportunistic pathogen in a tertiary care hospital setting

Tasneem Siddiqui, Sangram Singh Patel, Richa Sinha, Ujjala Ghoshal and Chinmoy Sahu*

Abstract

Introduction. *Ralstonia mannitolilytica* is a rare opportunistic pathogen capable of causing a serious infection in immunocompromised patients. Our objective was to describe all cases of *R. mannitolilytica* bloodstream infection identified within 2 years at our tertiary care centre, focusing on clinical characteristics, risk factors, antibiotic sensitivity patterns, management and outcomes.

Case Series. We compiled a descriptive case series including 14 non-duplicate *R. mannitolilytica* isolates obtained from bloodstream infection samples from the microbiology laboratory of a tertiary care centre from June 2019 to June 2021. All isolates were initially identified based on their morphological properties and biochemical reactions, and then underwent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) examination for confirmation of identity. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method and Vitek 2. All 14 patients presented with symptoms of fever and/or chills, and a positive blood culture for *R. mannitolilytica*. After 48 h of incubation, no *Ralstonia* growth was reported from any of the current environmental or pharmaceutical water samples. Chemotherapy (9/14), mechanical ventilation (4/14), steroid use (2/14) and diabetes mellitus (1/14) were associated risk factors in our patients. The antibiotic sensitivity panel showed maximum resistance to aminoglycosides (64.3%) and no resistance to cefoperazone/sulbactam. Patients received treatment with cefoperazone/sulbactam and meropenem or ceftazidime. Thirteen patients recovered with antibiotic therapy and one patient succumbed to his illness.

Conclusion. *R. mannitolilytica* can cause bloodstream infections in immunocompromised patients. It is likely to be missed or underreported due to lack of clinical awareness. MALDI-TOF MS is helpful in rapid identification. *R. mannitolilytica* is resistant to many routinely used antibiotics, including carbapenems.

INTRODUCTION

Ralstonia mannitolilytica – aerobic Gram-negative non-fermentative rods – are environmental organisms commonly found in water and soil, but are now emerging as opportunistic pathogens causing infections in immunocompromised patients [1]. They could be an aetiological agent in common source nosocomial outbreaks due to contamination of parenteral fluid and medical equipment that is considered to be sterile [2]. *R. mannitolilytica* are known to cause sepsis, meningitis and central venous catheter-associated bacteraemia [3]. Of late several case reports of infections by *R. mannitolilytica* have been seen from India and worldwide [4–12]. Many case reports have highlighted *R. mannitolilytica* as causing bacteraemia and sepsis [4–11]. Lampropoulos *et al.* (2021) and Rajendran *et al.* (2022) highlighted the organism as an emerging opportunistic pathogen causing sepsis in neonatal intensive care units [9, 10]. Carreira *et al.* (2020) emphasized the role of *R. mannitolilytica* in endocarditis [12]. The tendency of *R. mannitolilytica* to form biofilm enhances the organism's survival in the environment (including the hospital environment), and plays a role in frequent antibiotic resistance [13].

There are currently no clear treatment guidelines or Clinical and Laboratory Standards Institute (CLSI) breakpoints for *R. mannitolilytica*. Treatment is challenging, as this species is frequently resistant to many antibiotics [1]. Resistance to many of the

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Author affiliations: ¹Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

***Correspondence:** Chinmoy Sahu, sahu.chinmoy@gmail.com

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Abbreviations: AST, antibiotic sensitivity testing; CLSI, Clinical and Laboratory Standards Institute; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

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β -lactam class of antibiotics, including the carbapenems, is generally observed in *R. mannitolilytica*. blaOXA-22 and blaOXA-60 are class D carbapenemase genes that are commonly associated with *Ralstonia* species [14]. Treatment is based on the antibiotic susceptibility profile of the isolate [1]. There are very few case reports on this rare opportunistic pathogen in bloodstream infections from India. In this case series, we summarize the clinical characteristics of 14 patients with nosocomial bloodstream infections caused by *R. mannitolilytica* in the last 2 years, and analyse their risk factors, management and outcomes. We have also reported drug susceptibility patterns, which may help in the management of patients infected with this bacterium.

CASE SERIES

This case series includes clinically significant non-duplicate culture isolates of *Ralstonia* species obtained from suspected bloodstream infections in microbiology laboratory of a tertiary care centre of northern India. These cases occurred between June 2019 and June 2021 in the Departments of Haematology, Critical Care medicine, Gastro-medicine, Pulmonary Medicine and Endocrine Surgery. Written informed consent for publication of clinical details was obtained from the patients before the study. A study proforma was designed, which included patient demographic data, clinical details, underlying risk factors during the episode of infection, duration of hospital stay, treatment received and outcomes.

Sample processing and identification of isolates

Blood samples were inoculated into aerobic and anaerobic blood culture bottles (Becton Dickinson Diagnostics, USA) and incubated in the BACTEC blood culture system (BD Diagnostics, USA). Once flagged positive, a Gram stain was performed from the bottle and the broth was plated onto 5% sheep blood agar and MacConkey agar. The plates were incubated at 37 °C in ambient air and then inspected for growth at 24 h and again at 48 h. All of the culture media used were obtained from HiMedia Laboratories (Mumbai, India). The bacterial isolates were first identified using the routine staining and biochemical tests used in our laboratory [15]. The biochemical reactions for this Gram-negative bacillus revealed the results as follows: catalase-positive, oxidase-positive, motile, non-fermenting, methyl red-negative, Voges-Proskauer-negative, indole-negative, triple sugar iron agar – K/K (alkaline/alkaline), and urease-negative. Citrate was utilized but aesculin and gelatin were not hydrolyzed. *R. mannitolilytica* and *Ralstonia pickettii* were differentiated on the basis of nitrate reduction (negative in *R. mannitolilytica*) and acidification of D-arabitol and mannitol (both negative in *R. pickettii*) [16]. The identity of all the isolates was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the VITEK MS system (bioMérieux, Marcy-l'Etoile, France). Briefly, one bacterial colony of each isolate was spotted directly onto a single well of a disposable, barcode-labelled Vitek MS-DS target slide (bioMérieux, Marcy l'Etoile, France) and overlaid with 1 μ l of saturated α -cyano-4-hydroxycinnamic acid (CHCA) (Vitek MS-CHCA, bioMérieux, Marcy l'Etoile, France) matrix and then air-dried. *Escherichia coli* ATCC 8739 was used as the quality control strain and was transferred directly to designated spots on the target slide as per the recommendation of the manufacturer. The target slide with all prepared isolates was then loaded into the VITEK MS system to acquire the mass spectra of bacterial proteins [17]. Finally, the mass spectra acquired for each isolate were compared to the known mass spectra contained in the SARAMIS database. The software compares the spectra and generates a numerical value (score value) based on the similarities between the observed and stored data sets. A score value above 2.0 is generally considered to be a valid species level identification and values between 2.0 and 1.7 represent reliable genus-level identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Müller-Hinton agar and with an automated method (Vitek 2, bioMérieux) [18]. Antibiotic sensitivity was tested as per the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. As there were no CLSI breakpoints or zone diameters available for *R. mannitolilytica*, the results were interpreted using the CLSI breakpoints for *Pseudomonas* spp. [19]. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were put up as controls.

Environmental surveillance sampling

Environmental samples were collected from different wards from which *R. mannitolilytica* isolates had been obtained by an infection control nurse. Commercially available sterile swabs (HiMedia Laboratories, Mumbai, India) were used to collect samples from the patients' immediate surroundings, bed rails, tubing and medical devices. Samples from unused sterile intravenous fluids, liquid soaps and disinfectants and water (drinking water and tap water) were obtained in a sterile universal container. Air sampling was performed using a sieve impactor.

Processing of environmental samples

Swabs were incubated in brain heart infusion (BHI) media at 37 °C for 18–24 h. After 24 h, BHI media was inspected visually for any turbidity or growth. A small volume of sample was then taken with the help of an inoculating loop and sub-cultured on blood and MacConkey agar. The culture plates were further incubated for 24 h at 37 °C. Any positive growth was further identified using Gram staining and appropriate biochemical tests.

Table 1. Demographic and clinical characteristics of patients

Patient no.	Age range	Clinical diagnosis	Risk factors
1	21–30	Dengue haemorrhagic shock syndrome	Gastrointestinal surgery within 30 days, mechanical ventilation, steroid use
2	51–60	Decompensated chronic liver disease	Diabetes mellitus
3	51–60	Acute respiratory distress syndrome	Mechanical ventilation, anti-tubercular treatment
4	31–40	Acute necrotizing pancreatitis	Mechanical ventilation
5	11–20	Acute lymphoblastic leukaemia	Chemotherapy
6	51–60	Severe COVID-19 pneumonia	Mechanical ventilation, steroids
7	11–20	Acute myeloid leukaemia	Chemotherapy
8	51–60	Breast carcinoma	Chemotherapy
9	11–20	Acute myeloid leukaemia	Chemotherapy
10	1–10	Hodgkin's lymphoma	Chemotherapy
11	11–20	Hodgkin's lymphoma	Chemotherapy
12	11–20	Acute myeloid leukaemia	Chemotherapy
13	11–20	Acute lymphoblastic leukaemia	Chemotherapy
14	11–20	Acute myeloid leukaemia	Chemotherapy

Statistical analysis

Statistical tests were performed using SPSS for Windows version 14 (SPSS, Inc., Chicago, IL, USA) for descriptive statistics. Categorical data were described using numbers and percentages.

RESULTS

A total of 14 cases of *R. mannitolilytica* were reported from our hospital over a period of 2 years. All of the patients presented with symptoms of fever and/or chills, and a positive blood culture for *R. mannitolilytica*. We carried out comprehensive environmental sampling from various sites in our hospital. However, after 48 h of incubation all of the cultures were found to be sterile. The age of the patient population ranged from 8 to 58 years with a median age of 15.5 years. The number of males and females enrolled in the study were 10 and 4, respectively, with a M : F ratio of 2.5 : 1. Detailed demographic and clinical characteristics of the patients are illustrated in Table 1. The majority of the patients with *R. mannitolilytica* infection had haematological malignancy (8/14) and there was one patient each with breast carcinoma, dengue haemorrhagic shock syndrome, decompensated chronic liver disease, acute respiratory distress syndrome, acute necrotizing pancreatitis and severe coronavirus disease 2019 (COVID-19) pneumonia. The average duration of hospital stay was 29 days (range 10–58 days) and the mean time of development of infection after hospitalization was 15.1 days. Chemotherapy (9/14), mechanical ventilation (4/14) and steroid (2/14) use were the most common risk factors in these patients.

MALDI-TOF-MS identified all 14 isolates as *R. mannitolilytica* with confidence values of 99.9%. All of the isolates (100%) were sensitive to cefoperazone/sulbactam (Fig. 1). It was determined that 64.3% (9/14) and 50% (7/14) of isolates were resistant to aminoglycosides (amikacin and gentamicin) and ceftazidime, respectively; 42.8% (6/14) of isolates were resistant to each of piperacillin/tazobactam, cotrimoxazole and carbapenems (imipenem and meropenem); and 21.4% (3/14) and 14.3% (2/14) of isolates were resistant to fluoroquinolones (ciprofloxacin and levofloxacin) and cefepime, respectively.

The following antibiotics were administered to patients after antibiotic susceptibility testing and according to the clinicians' decisions: cefoperazone/sulbactam was given to 10 (71.4%) patients, meropenem to 3 (21.4%) patients and ceftazidime to 1 (7.2%) patient. Thirteen patients recovered with antibiotic therapy and one patient succumbed to his illness, but the cause of death for this patient was complications of COVID-19 (Table 2).

DISCUSSION AND CONCLUSION

Few cases of infections caused by *R. mannitolilytica* have been reported due to limited awareness of the pathogen. The development of modern medical care, inappropriate and unnecessary use of broad-spectrum antibiotics, and the extensive use of various immunosuppressants have caused increased rates of opportunistic infections from organisms such as *R. mannitolilytica*. The

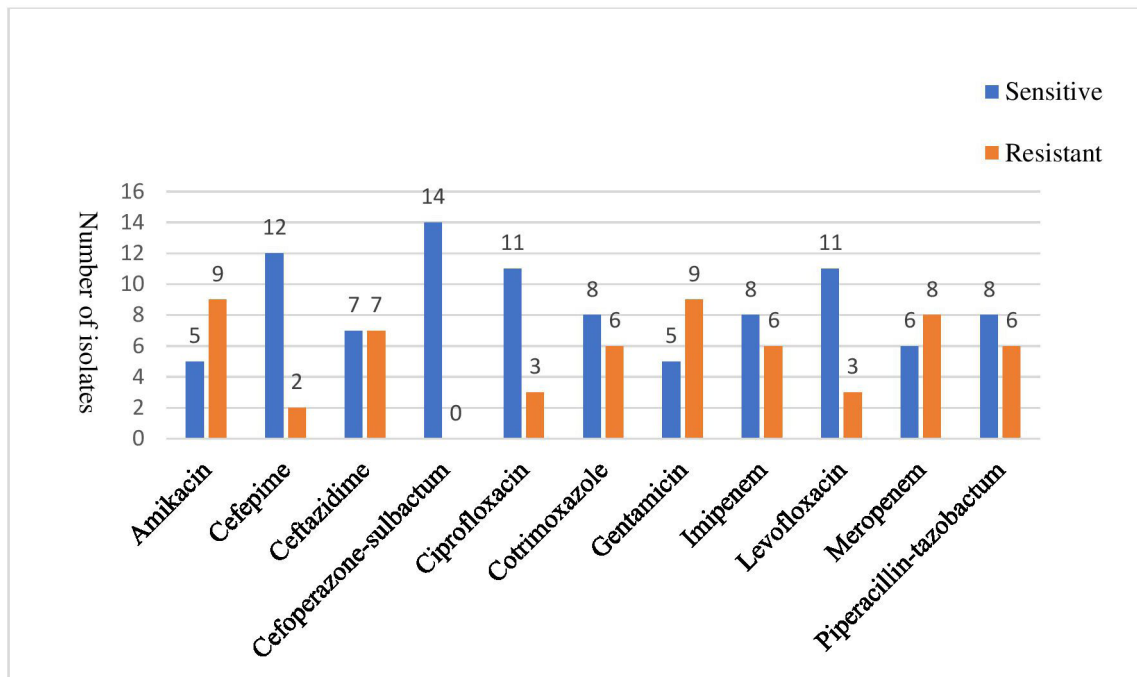


Fig. 1. Antimicrobial susceptibility of 14 clinical isolates of *R. mannitolilytica* determined using the CLSI standards and interpreted as per the CLSI breakpoints for *Pseudomonas* spp.

prevalence of *Ralstonia* infection is increasing notably, even without person-to-person transmission [1]. *Ralstonia* exist widely in external aqueous environments, including municipal water and medical water purification systems [20, 21]. As the bacteria can pass through 0.2 μm filters during the sterilization process, medical products may be contaminated during the manufacturing phase [22]. *Ralstonia* can create biofilms on the surfaces of medical supplies and produce toxins [13]. Most infectious cases caused

Table 2. Timeline of infection, identification, management and outcome for the patients

Patient no.	Duration of hospital stay (days)	Time from admission to development of infection (days)	Identity of isolates (MALDI-TOF)	Treatment received	Outcome/follow-up
1	11	5	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
2	15	11	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
3	58	27	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
4	35	5	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
5	10	8	<i>R. mannitolilytica</i>	Meropenem+colistin	Recovered
6	49	26	<i>R. mannitolilytica</i>	Meropenem+colistin	Dead
7	20	8	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
8	10	6	<i>R. mannitolilytica</i>	Meropenem+colistin	Recovered
9	21	6	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
10	40	30	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
11	29	25	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
12	52	32	<i>R. mannitolilytica</i>	Ceftazidime+amikacin	Recovered
13	31	12	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered
14	28	10	<i>R. mannitolilytica</i>	Cefoperazone/sulbactam	Recovered

by *Ralstonia* species are due to the use of contaminated solutions, chlorhexidine, saline solution, blood products and sterile water as well as the colonization of medical devices (tap water and water used for haemodialysis, bronchoscope flushing and heparin for flushing) [3, 23, 24].

In this case series we have described 14 nosocomial cases of bloodstream infections caused by *R. mannitolilytica*, although we could not track their source. All of our patients were at high risk of infection. The high-risk factors listed in the literature are cancer, blood vessel catheters, mechanical ventilation and other immunocompromised conditions [1, 25] that were also found in our study. The most frequent risk factor in the study population was the use of chemotherapy and steroids, which could be why most of the patients in the study had malignancy, which itself is an immunocompromised state [3]. Further, these patients received chemotherapy as well as immunosuppressant drugs [3]. Hence, such patients are vulnerable to opportunistic infections such as *Ralstonia*, which is non-harmful to healthy persons [1]. Long-term use of steroids also causes an increase in the rate of infections due to deranged cellular immunity. Central lines are essential in malignancy patients for long-term chemotherapy infusions, and infection of central lines might happen during the insertion procedure as well as during the maintenance period. Biofilm formation in the central lines might cause bacteria to harbour and cause central line-associated bloodstream infections [4]. Blood products through central lines might help in the formation of biofilms that might cause central line infections in these cases. It is well accepted that, over time, the presence of endotracheal tubes for mechanical ventilation increases the risk of bacterial colonization and the development of infection. Similarly, it is conceivable that *Ralstonia*, a water-borne bacterium cultured from respiratory devices, may have the capacity to colonize the airways; its capacity to generate biofilms, like *Pseudomonas* species, enables it to cause infection in patients who are mechanically ventilated for longer periods [21]. Diabetes itself causes increased infections due to unbalanced blood sugar levels in patients. All of these patients had prolonged hospital stays, which might have led to acquisition of this bacterium. Boattini *et al.* (2018) reported that this pathogen is an important cause of nosocomial bacteraemia in diabetics, preterm infants, solid organ and haematological malignancy patients, and patients with end-stage renal disease [4]. Thomas *et al.* (2021) reported it to cause bacteraemia and gastroenteritis in a patient with rheumatoid arthritis.

Diagnosis and management of *Ralstonia* spp. infections is challenging. First, there is the difficulty of correctly identifying and differentiating between *Ralstonia* spp. members using routine laboratory analyses, because they have very similar biochemical patterns to each other and to other bacterial genera, such as the *Burkholderia cepacia* complex [1]. MALDI-TOF showed good performance regarding the identification of *R. mannitolilytica* in a previous study using isolates from patients with cystic fibrosis [26]. Although 16S rDNA is the reference method for identifying micro-organisms, it is costly and cumbersome. In the present study, MALDI-TOF correctly identified all of the isolates rapidly. Therefore, in comparison to the turnaround time and PCR identification cost of the 16S sequencing method, MALDI-TOF MS would be a better choice for identification of *R. mannitolilytica*. Our results support the general consensus that MALDI-TOF MS can provide rapid and accurate results [27]. Second, *R. mannitolilytica* are frequently resistant to numerous different types of antibiotics, including several beta-lactams and most of the aminoglycosides [1]. The organism may produce various enzymes that can hydrolyze antibiotics. These can confer resistance to a broad range of antibiotics, including benzylpenicillin, narrow-spectrum cephalosporins, ceftazidime, aztreonam and the carbapenems [26]. As currently there are no clear treatment guidelines for *R. mannitolilytica* and the data from various case reports reveal a notable heterogeneity in the percentage of antibiotic resistance amongst *R. mannitolilytica* isolates, in the course of treatment we advocate using antibiotic susceptibility testing to adjust the use of antimicrobial agents. Our antibiotic susceptibility pattern showed maximum resistance to aminoglycosides and no resistance to cefoperazone/sulbactam. Hence most of our patients were treated with cefoperazone/sulbactam or a with some other third-generation cephalosporins. Daxboeck *et al.* reported carbapenem resistance in 12 out of their 30 strains [28], which is consistent with our study, where we found carbapenem resistance in 6 out of 14 isolates. Thus meropenem was only used for seriously ill patients whose antibiograms showed sensitivity to meropenem. A 93% (13/14) recovery rate in the study patients supports the view that early diagnosis and early initiation of appropriate antibiotics are required for good outcomes in these patients.

Even though *R. mannitolilytica* is not recognized as a major pathogen, clinicians and microbiologists should pay attention to the potential of this opportunistic bacterium, which is able to cause bloodstream infections, as it has certain notable characteristics, such as multidrug resistance, the ability to survive in water supplies and resistance to disinfection practices. Prompt diagnosis and subsequent administration of antibiotics in line with antimicrobial susceptibility testing results are needed to clear infections. MALDI-TOF MS is helpful for rapid identification and *R. mannitolilytica* is capable of being resistant to many routinely used antibiotics, including carbapenems.

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Author contributions

T.S., writing – original draft; S.S.P., methodology; U.G., supervision; R.S., resources, data curation; C.S., conceptualization, writing – review and editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Ethical approval was obtained from institutional ethics committee.

Consent to publish

Written informed consent for publication of clinical details was obtained from the patients.

References

- Ryan MP, Adley CC. *Ralstonia* spp.: emerging global opportunistic pathogens. *Eur J Clin Microbiol Infect Dis* 2014;33:291–304.
- Mukhopadhyay C, Bhargava A, Ayyagari A. *Ralstonia mannitolilytica* infection in renal transplant recipient: first report. *Indian J Med Microbiol* 2003;21:284–286.
- Lucarelli C, Di Domenico EG, Toma L, Bracco D, Prignano G, et al. *Ralstonia mannitolilytica* infections in an oncologic day ward: description of a cluster among high-risk patients. *Antimicrob Resist Infect Control* 2017;6:20.
- Boattini M, Bianco G, Biancone L, Cavallo R, Costa C. *Ralstonia mannitolilytica* bacteraemia: a case report and literature review. *Infez Med* 2018;26:374–378.
- Pérez Lazo G, Silva Caso W, Morales Moreno A, Soto Febres F, Morales Castillo L, et al. Bacteremia due to *Ralstonia mannitolilytica*: A report of the first case in Peru. *Medwave* 2021;21:e8200.
- Thomas KN, Sahu C, Aggarwal A. *Ralstonia mannitolilytica* bacteraemia and gastroenteritis in a patient with rheumatoid arthritis: an emerging nosocomial infection. *Rheumatology (Oxford)* 2021;60:e195–e196.
- Owusu M, Acheampong G, Annan A, Marfo KS, Osei I, et al. *Ralstonia mannitolilytica* sepsis: a case report. *J Med Case Rep* 2019;13:318.
- Zhou S, Tang D, Wei S, Hu Z, Wang X, et al. *Ralstonia mannitolilytica* sepsis after elective cesarean delivery: a case report. *BMC Pregnancy Childbirth* 2021;21:737.
- Lampropoulos P, Gkentzi D, Tzifas S, Kapnisi G, Karatza A, et al. *Ralstonia mannitolilytica*, an unusual pathogen in the neonatal intensive care unit: a case of neonatal sepsis and literature review. *Infect Disord Drug Targets* 2021;21:168–172.
- Rajendran UD, Sundaramoorthy S, Sethuraman G. *Ralstonia mannitolilytica* sepsis in neonatal intensive care unit - Be(a)ware of the multidrug resistant nosocomial bug. *Trop Doct* 2022;52:216–217.
- Sriwijitalai W, Wiwanitkit V. *Ralstonia* infection among hemodialysis patients: A summary. *Saudi J Kidney Dis Transpl* 2019;30:749–750.
- Carreira M, Gomes C, Silva M, Duro R. *Ralstonia mannitolilytica* endocarditis: A case report. *IDCases* 2020;22:e01003.
- Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiol Mol Biol Rev* 2014;78:510–543.
- Basso M, Venditti C, Raponi G, Navazio AS, Alessandr F, et al. A case of persistent bacteraemia by *Ralstonia mannitolilytica* and *Ralstonia pickettii* in an intensive care unit. *Infect Drug Resist* 2019;12:2391–2395.
- Collee JG, Mackie TJ, McCartney JE. Processing of samples. In: *Mackie & McCartney Practical Medical Microbiology*, 14th ed. New York: Churchill Livingstone, 1996.
- De Baere T, Steyaert S, Wauters G, Des Vos P, Goris J, et al. Classification of *Ralstonia pickettii* biovar 3/'thomasii' strains (Pickett 1994) and of new isolates related to nosocomial recurrent meningitis as *Ralstonia mannitolilytica* sp. nov. *Int J Syst Evol Microbiol* 2001;51:547–558.
- BioMérieux. VITEK EXPANDED DATABASE MS Now with Mycobacteria, Nocardia and Moulds; 2016. https://www.biomerieux-diagnostics.com/sites/clinic/files/vitek_ms_brochure_9312647_002_gb_a_web.pdf
- Ling TK, Tam PC, Liu ZK, Cheng AF. Evaluation of VITEK 2 rapid identification and susceptibility testing system against gram-negative clinical isolates. *J Clin Microbiol* 2001;39:2964–2966.
- Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing. CLSI Supplement M-100*. 30th ed. Wayne, PA: CLSI; 2020.
- Chen Y-Y, Huang W-T, Chen C-P, Sun S-M, Kuo F-M, et al. An outbreak of *Ralstonia pickettii* bloodstream infection associated with an intrinsically contaminated normal saline solution. *Infect Control Hosp Epidemiol* 2017;38:444–448.
- Vincenti S, Quaranta G, De Meo C, Bruno S, Ficarra MG, et al. Non-fermentative gram-negative bacteria in hospital tap water and water used for haemodialysis and bronchoscope flushing: prevalence and distribution of antibiotic resistant strains. *Sci Total Environ* 2014;499:47–54.
- Anderson RL, Bland LA, Favero MS, McNeil MM, Davis BJ, et al. Factors associated with *Pseudomonas pickettii* intrinsic contamination of commercial respiratory therapy solutions marketed as sterile. *Appl Environ Microbiol* 1985;50:1343–1348.
- Tejera D, Limongi G, Bertullo M, Cancela M. *Ralstonia pickettii* bacteremia in hemodialysis patients: a report of two cases. *Rev Bras Ter Intensiva* 2016;28:195–198.
- Lai H-W, Shen Y-H, Chien L-J, Tseng S-H, Mu J-J, et al. Outbreak of *Ralstonia pickettii* bacteremia caused by contaminated saline solution in Taiwan. *Am J Infect Control* 2016;44:1191–1192.
- Waugh JB, Granger WM, Gaggari A. Incidence, relevance and response for *Ralstonia* respiratory infections. *Clin Lab Sci* 2010;23:99–106.
- Prior AR, Gunaratnam C, Humphreys H. *Ralstonia* species - do these bacteria matter in cystic fibrosis? *Paediatr Respir Rev* 2017;23:78–83.
- Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, et al. Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. *J Clin Microbiol* 2008;46:1946–1954.
- Daxboeck F, Stadler M, Assadian O, Marko E, Hirschl AM, et al. Characterization of clinically isolated *Ralstonia mannitolilytica* strains using random amplification of polymorphic DNA (RAPD) typing and antimicrobial sensitivity, and comparison of the classification efficacy of phenotypic and genotypic assays. *J Med Microbiol* 2005;54:55–61.