Case Report

**Ralstonia mannitolilytica bacteremia in a neonatal intensive care unit**

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**Abstract**

*Ralstonia mannitolilytica*, a Gram-negative bacterium, is rarely isolated in clinical laboratories. It has been associated with outbreaks due to its ability to survive in liquid media and hospital devices. We describe three cases of bacteremia caused by *R. mannitolilytica* in a neonatal intensive care unit in Curitiba, Southern Brazil. All isolates presented the same PFGE profile. The common source of infection was undetected in surveillance cultures for the outbreak survey. All patients received antimicrobial treatment and were discharged from the maternity. Due to the characteristics of the microorganism, clinicians and microbiologists should pay attention to the emergence of *Ralstonia* spp. infections.

**Keywords**: *R. mannitolilytica*. Bacteremia. *Ralstonia* spp.

**INTRODUCTION**

*Ralstonia mannitolilytica* is a non-fermenting, Gram-negative bacterium[1]. Sixteen species and three subspecies belong to the genus *Ralstonia*, and they have adapted to thrive under low-nutrient conditions, such as in the presence of disinfectants and in various water sources[1]. Clinical conditions associated with *Ralstonia* spp., though rare, range from minor respiratory infections to severe invasive infections, such as sepsis and meningitis[1].

The frequency of infections due to *Ralstonia* spp., and in particular *R. mannitolilytica*, is not widely reported. Therefore, this study aimed to describe three cases of bacteremia due to *R. mannitolilytica* in a neonatal intensive care unit (NICU) at a maternity hospital located in Curitiba, Southern Brazil.

**CASE REPORT**

The first patient, a newborn male, with a birth weight of 3,690g and without intercurrences during the hospital stay, was discharged from hospital 48 hours after birth. However, he was readmitted to the hospital 23 days later (March 5, 2015) with vomiting, hypovolemic shock, acute respiratory failure, and late-onset sepsis. The patient was submitted to mechanical ventilation in the NICU. His white blood cell (WBC) count was 33,310/μL with 11% bands, and a blood culture yielded positive results for Gram-positive cocci and Gram-negative bacilli. The patient received meropenem, vancomycin, and cefepime. Then, the bacteria were identified as *R. mannitolilytica* and coagulase-negative *Staphylococcus*. *R. mannitolilytica* was isolated from three more blood culture samples during the sequent days. In the samples collected on March 8, the blood cultures yielded negative findings. On March 11, the patient was transferred to a pediatric hospital.

The second patient, a female born premature and with a low weight of 1,450g on March 3 and was transferred to the NICU. On March 6, her WBC count was 6,360/μL with 1% bands and 1% metamyelocytes, and a blood culture yielded Gram-negative bacilli. The patient received meropenem and cefepime. Then, the bacteria were identified as *R. mannitolilytica* and coagulase-negative *Staphylococcus*. *R. mannitolilytica* was isolated from three more blood culture samples during the sequent days. In the samples collected on March 8, the blood cultures yielded negative findings. On March 11, the patient was transferred to a pediatric hospital.

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The third patient, a male newborn, weighing 2,375g at birth, was transferred to the NICU on March 4, owing to low weight. On the same day, a complete blood count showed a WBC count of 20,290/μL with 5% bands, and a blood culture yielded negative findings. On March 16, the WBC count was 4,360/μL with 1% bands, and the blood culture yielded positive
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results. The bacterium was identified as *Escherichia coli*. On March 24, a complete blood count showed a WBC count of 13,000/μL with 4% bands and 2% myelocytes, and the blood culture yielded positive results. The bacterium was identified as *R. mannitolilytica*. The patient received cefepime, vancomycin, and meropenem during the hospital stay. On March 30, he was discharged from the hospital.

An outbreak investigation was initiated at the institution. Surveillance cultures (nasal and oropharynx swabs) were collected from the other 9 newborns in the NICU at the time of the outbreak. Samples of different parenteral solutions administered to the newborns with *R. mannitolilytica* (50% glucose, 5% glucose, potassium chloride, 20% sodium chloride, 0.9% sodium chloride, and water for injection), environmental samples, and liquid soap were also collected for research on this bacterium. However, it was not found in any of the analyzed samples.

In the laboratory analysis, the blood cultures were inoculated into a pediatric bottle and incubated in BD BACTECTM FX (BD Diagnostic Systems, New Jersey, USA). After positive detection, samples were subcultured on chocolate agar and incubated for 24h at 37°C. Isolates were identified using a VITEK 2 Compact (BioMérieux S.A., Marcy l’Etoile, France), complementary phenotypic tests, mass spectrometry (Microflex LT; Bruker Daltonics, Bremen, Germany), and 16S ribosomal deoxyribonucleic acid (rDNA) gene sequencing. Antimicrobial susceptibility was tested for 22 antimicrobial agents by broth microdilution using plates of Sensititre™ (Thermo Fisher Scientific, MA, USA) containing Gram-negative bacteria. The minimum inhibitory concentration obtained were the same for all the *R. mannitolilytica* isolates: piperacillin >64µg/mL, piperacillin/tazobactam >64/4µg/mL, ticarcillin/ clavulanate >64/2µg/mL; ceftazidime >16µg/mL, cefepime 16µg/mL, aztreonam >16µg/mL, meropenem >8µg/mL, imipenem >8µg/mL, doripenem >4µg/mL, trimethoprim- sulfamethoxazole <2/38µg/mL, gentamicin >8µg/mL, amikacin >32µg/mL, levofloxacin <1µg/mL, tetracycline >8µg/mL, minocycline 4µg/mL, and tigecycline <1, even though there are no standardized criteria available for laboratories to interpret the susceptibility testing for *Ralstonia* spp.

The genetic similarity of the isolates of *R. mannitolilytica* was compared using macrorestriction [XbaI (10U)] analysis by pulsed-field gel electrophoresis (PFGE) (CHEF-DRIII; Bio-Rad Laboratories, USA). The PFGE profiles were analyzed by comparison with the standards using a Gel-Pro Analyzer 4.0 and NTSYS software to calculate the Dice similarity coefficient and to implement the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to create dendrograms (Figure 1). The Tenover criteria were used to define the genetically related isolates4.

**DISCUSSION**

*Ralstonia mannitolilytica* is a gram-negative bacillus that had previously been named *Pseudomonas thomasii* and *Ralstonia picketti* biovar 3/”thomasii” and is rarely isolated in clinical laboratories. It has been associated with outbreaks, owing to its low nutritional need and ability to survive in liquid media and hospital devices. With the global emergence of this bacterium, there have been reports of bacteremia in the literature1. In this report, we described 3 cases of bacteremia that occurred in a maternity hospital; the first case describes the first incidence of sepsis associated with this microorganism.

Although the 16S rDNA is the reference method for identifying microorganisms, in this study, the Vitek system and MALDI-TOF (Matrix-assisted laser desorção / ionização Time of Flight) correctly identified the three isolates. It is difficult to identify and differentiate between *Ralstonia* spp. using routine hospital analyses, because they have very similar biochemical patterns among each other and with other bacterial genera, such as the *Burkholderia cepacia* complex1. MALDI-TOF had good performance regarding the identification of *R. mannitolilytica* in a previous study using isolates from patients with cystic fibrosis2.

The antimicrobial treatment and management of *Ralstonia* spp. infections are often challenging, as these pathogens are frequently resistant to numerous different types of antibiotics,

![FIGURE 1: Similarity analysis of Ralstonia mannitolilytica isolates. P1H1 - patient 1/blood sample 1; P1H2 patient 1/ blood sample 2; P1H3 patient 1/blood sample 3; P1H4: - patient 1/blood sample 4; P2, Patient 2; P3: patient 3.](image)
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\(^2\). The main mechanism that has been described for \textit{Ralstonia} spp. is a class D beta-lactamase. In a recent report, the first draft genome sequence of \textit{R. mannitolilytica} identified the \textit{bla}_{OXA-443} and \textit{bla}_{OXA-444} genes\(^5\). Other resistance mechanisms, such as an extended-spectrum beta-lactamase (ESBL), efflux pumps, \textit{qnr} genes, and ribosomal methyltransferases, have also been related.

In this study, all the isolates showed MICs below the lowest concentration tested for the following antimicrobials: levofloxacin, trimethoprim-sulfamethoxazole (co-trimoxazole), and tigecycline, but it is important to emphasize that there are no standardized criteria for the interpretation of susceptibility tests for \textit{Ralstonia} spp. Quinolones and co-trimoxazole have been suggested as the best agents for the treatment of an infection due to \textit{R. insidiosa} and \textit{R. pickettii}. These recommendations are based on \textit{in vitro} studies, and further studies are needed to assess the \textit{in vivo} effectiveness. However, the potential toxicity of quinolones needs to be considered in children\(^5\). The antimicrobial tigecycline has been shown in small studies to have good \textit{in vitro} activity against \textit{Ralstonia} spp.\(^2\), but this antimicrobial is not indicated in cases of bloodstream infection.

In addition to the multidrug resistance presented, other risk factors contribute to the acquisition of a \textit{Ralstonia} spp. infection, like the potential for biofilm formation\(^6\) and its ability to pass through 0.2-μm filters, which are used for the sterilization of many medical products\(^1\).

All the patients achieved clinical improvement, and despite the efforts of the hospital staff, the source of infection was not found in this report. The time between the first case and the last case (15 days) suggests a reservoir as the source of the cases. Likewise, the molecular typing of the pathogen strongly supports the hypothesis of a common source of contamination. Some studies have shown evidence regarding patients with shared strains, but inter-patient transmission has not been well documented until now, suggesting that a common reservoir among multiple patients may serve to promote outbreaks\(^7\).

Although \textit{Ralstonia mannitolilytica} is not recognized as a major pathogen, clinicians and microbiologists should pay attention to this species, which has certain characteristics, such as multidrug resistance, the ability to survive in water supplies, and resistance to disinfection practices, that allow it to cause many, potentially lethal infections.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

**REFERENCES**