**Ralstonia mannitolilytica** Bacteremia in an Immunocompromised Patient: Case Report and Review

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**ABSTRACT:** *Ralstonia mannitolilytica* is an emerging opportunist pathogen reported in many healthcare facilities over the years. We report a case with *R. mannitolilytica* bacteraemia in breast carcinoma patient with chemo port. Identification of this non fermentative, Gram negative bacilli was done by Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI TOF MS). A minireview of cases of *R. mannitolilytica* bacteraemia in the recent years with special reference to those reported in India is done.

**KEYWORDS:** Chemo Port, Central Venous Catheter, MALDI-TOF, Opportunistic Pathogen, *R. mannitolilytica* Bacteraemia.

**INTRODUCTION**

The genus *Ralstonia* comprises a group of non-fermentative, Gram-negative bacteria (NFGN) found in moist environments, such as water, soil, and plants [1]. The genus *Ralstonia* has six species, of which *Ralstonia pickettii, Ralstonia insidiosa* and *Ralstonia mannitolilytica* have been recognized as opportunistic human pathogens [1]. Their relevance has been currently re-evaluated because of their ability to survive in different types of disinfectants and to pass through 0.2 μm filters that are used to sterilize solutions [1, 2]. Multidrug resistance in NFGN is widely reported in the literature and is causing increasing concern because such bacteria may have a role not only as human pathogens but also as potential reservoirs of resistance genes, particularly when they are found in hospital settings [1]. Clinical isolation of *Ralstonia* is rare in India and thus the lack of sufficient experience with its diagnosis and treatment. A case of *R. mannitolilytica* bacteraemia in post operative carcinoma breast patient is presented here along with a review of *R. mannitolilytica* bacteraemia cases and outbreaks reported in recent years to highlight the clinical, diagnostic, prognostic, and microbiologic features of this emerging pathogen for its better management in Indian setup.

**CASE**

A 38 years old female patient was admitted with history of one day high grade fever. She was a diagnosed case of a right sided carcinoma breast stage II and had undergone modified radical mastectomy six months before. She had one indwelling chemo port for adjuvant combination chemotherapy since four months. Patient had developed fever with chills one day after her fourth chemotherapy cycle completion. She appeared toxic, with temperature 102.8°F, pulse rate 106 /min, respiratory rate 28/min and blood pressure 100/60 mm Hg. Systemic examination was unremarkable. Investigations revealed a total count of 15,763/mm³, neutrophils of 92%, haemoglobin of 9 g/dl, platelet count of 229,000/mm³. C-reactive protein was 9.1 mg/dL. Liver function tests, Renal function tests and urine routine examination did not show any significant abnormality. Chest-X ray showed no lung infiltration. Abdominal ultrasound revealed no alterations suggestive for infectious foci. Two sets of blood cultures were taken from the peripheral vein and chemo port. She was then put on Piperacillin-tazobactam empirically, but fever spikes persisted.

After 24 hours of incubation in automated blood culture system (BD BACTEC™ FX Instrument, Becton Dickinson, USA), blood culture bottles flagged positive for growth. The differential time to positivity (DTP) between blood taken from chemo port and the peripheral vein was 6 hours 35 minutes. The Gram stain smears from blood culture bottles showed Gram negative, slender bacteria. Catheter tip of chemo port was processed by rolling the tip back and forth on the surface of a Columbia agar plate supplemented with 5% sheep blood, essentially as described by Maki DG, et al [3]. After 24 hours of incubation at 37°C, on sheep blood agar colonies were non hemolytic, small pinpoint, opaque, circular, and convex. Non lactose fermenting, small, convex colonies were non hemolytic, small pinpoint, opaque, circular, and convex.

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observed on MacConkey agar. The isolate was motile, catalase and oxidase positive. On further biochemical testing, glucose was oxidized, urea was hydrolysed and nitrates were not reduced to nitrites. It was presumptively reported as Gram negative Nonfermenter as it was unidentified by Vitek 2 (bioMérieux, France). Subsequently, it was identified as Ralstonia mannitolilytica by Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI TOF MS) (bioMérieux, France) with 99.9% confidence value. Diagnosis of catheter related blood stream infection (CRBSI) caused by Ralstonia mannitolilytica was made. Piperacillin-tazobactam was discontinued and Imipenem was added to the treatment protocol.

Antimicrobial susceptibility testing by broth microdilution was done and interpreted as per Clinical and Laboratory Standards Institute (CLSI) M100 recommendations for ATCC Pseudomonas aeruginosa 27853. The isolate was found to be susceptible to Imipenem (MIC 4 μg/mL), Cefepime (MIC 4 μg/mL) and Cefoperazone/ Sulbactam (MIC ≤8 μg/mL). Resistance was noted for Ticarcillin/Clavulanic acid (MIC ≥128 μg/mL), Piperacillin/Tazobactam (MIC ≥128 μg/mL), Amikacin (MIC ≥ 64 μg/mL), Gentamicin (MIC ≥ 16 μg/mL), Ciprofloxacin (MIC ≥ 4 μg/mL), Levofloxacin (MIC ≥ 4 μg/mL) Colistin (MIC ≥ 16 μg/mL) and Intermediate susceptibility was observed for Ceftazidime (MIC 16 μg/mL). Based upon the sensitivity pattern observed, Imipenem was continued in treatment protocol. Two follow-up blood cultures were collected in the subsequent week which were negative for any bacterial growth. The patient recovered 10 days after starting therapy and bacteraemia due to the same pathogen had not recurred for more than six months.

To elucidate a source of R. mannitolilytica infection and to avoid outbreaks, a comprehensive environmental sampling was done including from in-use parental solutions, filled syringes, disinfectants, medical devices and water in the wards to which the patient had been admitted. Swabs were cultured in Tryptic Soy Broth, incubated for 48 hours at 37°C, and plated on chocolate agar and blood agar but yielded negative results. All microbiological data of hospital were reviewed, but no Ralstonia spp. have been matched in the last two years.

DISCUSSION
Nonfermenting Gram-negative rods are one of the commonest causes of nosocomial infections in clinical environments. The major opportunistic pathogens in this group are Acinetobacter baumannii; Stenotrophomonas maltophilia and other oxidase-positive bacteria such as Pseudomonas aeruginosa and Burkholderia cepacia [4]. R. mannitolilytica is another emerging opportunistic pathogen which was previously referred as Pseudomonas thomassii and R. picketii biovar 3/thomassii [4]. It has been reported in nosocomial outbreaks secondary to medical devices, equipment, water, or parenteral solutions contamination [5, 6]. It has been isolated in newborns and in patients with solid cancer, hematological disease, ventriculostrial draining for hydrocephalus, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and scleroderma [7]. Globally, the first reported outbreak of R. mannitolilytica was of 30 patients from USA in 2005 [8]. Since then, many outbreaks and cases have been reported. In India, very few cases of bacteremia with R. mannitolilytica have been reported. The first case reported in India was in a renal transplant patient by Mukhopadhyay et al in 2003 [9].

R. mannitolilytica grows readily on routine culture media i.e. trypticase soy agar with 5% sheep blood or Mac Conkey agar. However, when both biochemical tests and automated identification systems are used, Ralstonia spp. can be misidentified as Burkholderia spp. or non-aeruginosa Pseudomonas spp. [10]. R. mannitolilytica can be differentiated from Pseudomonas spp. and Burkholderia spp. by arginine dihydrolase test and pyrrolidonyl peptidase test [11]. The diagnostic methods used for identification are either ViTek 2 system with 16sRNA gene sequencing (molecular methods), PFGE or MALDI-TOF [12]. We identified Ralstonia mannitolilytica by MALDI TOF MS.

The most important source of infection is contaminated medical products during the manufacturing phase as the bacteria can pass through 0.2 μm filters during the sterilization process [1]. Colonization of medical devices like hemodialysis machine, bronchoscope, etc. and contamination of tap water, sterile water, saline solution, etc. are also major reasons for infections cases caused by Ralstonia spp. [11, 13]. Use of contaminated solution leading to biofilm formation which allowed adherence to central venous catheter (CVC) followed by its dissemination during the flushing process might be a possible cause of infection. In the cases reported by Lucarelli et al, Lim et al and Boattini et al, CVC was found to be the source of infection [5,13,14]. Whereas, in the study by Shankar et al the use of sterile water for IV drug preparation was the culprit [12]. Said M et al reported water in the dialysis system as the source of R. mannitolilytica [15]. In our case, as all the samples from disinfectants, antiseptics and saline solutions
were not available for microbiological investigation when the isolate was identified, we probably missed the exact source of infection. However, vigilant monitoring for successive months prevented further cases. The comprehensive minireview of literature of *R. mannitolilytica* bacteraemia in recent years was performed and depicted in the table 1 which shows demographic, clinical, diagnostic and prognostic features of 84 cases of *R. mannitolilytica* bacteraemia. No age or gender predilection was found. *R. mannitolityca* bacteraemia presents with symptoms of sepsis like any other pathogenic organism i.e. high grade fever, chills and neutrophilic leukocytosis. Majority of the patients were neonates, immunodeficient with frequent hospital visits or indwelling devices [4,5,10, 12-23].

*R. mannitolilytica* is known to have multidrug resistance although carbapenem resistance is not reported enough [24]. A combination of ciprofloxacin and trimethoprim-sulfamethoxazole is considered as the first-choice antibiotics in the treatment of *R. mannitolilytica* infection. Other treatment recommendations include third- generation cephalosporins or carbapenems [18]. In a case of infective endocarditis by *R. mannitolilytica*, carbapenems were found resistant and isolate was susceptible to only ciprofloxacin and co-trimoxazole. After two weeks of therapy, ciprofloxacin was found resistant thus showing the capacity of the organism to acquire resistance [19]. The isolate in our case was found susceptible to imipenem, cefepime and cefoperazone/ sulbactam. Similar susceptibility pattern was reported by Souza DC et al and Zhou S et al [17,20]. On the contrary, the strains identified in oncology ward of Italy were resistant to cefazidime, meropenem, fluoroquinolones, aminoglycosides but were susceptible to piperacillin/tazobactum [13]. Certain studies did molecular testing for resistant genes. Lucarelli et al found the *R. mannitolilytica* strains to be having AmpC B-lactamase, OXA-443 and OXA-444 [13]. Whole Genome Sequencing (WGS) of *R. mannitolilytica* strain isolated in Basso et al had OXA-22, OXA-443 and OXA-444 genes [18]. Persistent fever even after adding antibiotics mandated removal of chemo port in some studies like Chitre et al [23]. This hints towards biofilm formation in the chemo port and the need towards checking long standing indwelling devices. Biofilm provides a protective environment which helps in evasion from bactericidal effects of the antibiotics. Although, reported isolates were multidrug resistant, it was found that cases of *R. mannitolilytica* bacteraemia show favorable prognosis as in our case and the review table (81/84 recovered).

It is now evident that there is increased incidence of *Ralstonia* infections in healthcare settings, particularly in vulnerable patients who need continuous IV access, hemodialysis, nebulisations, etc. This is a major concern especially for Indian setup is underreporting of such cases due to inability of routine microbiological methods to identify *Ralstonia spp.* and further emergence of multi-resistant strains of *R.mannitolilytica* add to the existing burden.

**Table 1** – Demographic, Clinical, Diagnostic and Prognostic Findings

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Age</th>
<th>Clinical presentation</th>
<th>Healthcare setting</th>
<th>Identification system</th>
<th>Source of infection</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu CX et al. 2016</td>
<td>China</td>
<td>3</td>
<td>Fever with chills, septic shock</td>
<td>Oncology ward</td>
<td>VITEK Compact-2 (bioMérieux Inc., Marcy L’Etèole, France), PFGE</td>
<td>Not found</td>
<td>Cotrimoxazole ,Ceftriazone, Tazocin</td>
<td>Recovered</td>
</tr>
<tr>
<td>Lucarelli et al. 2017</td>
<td>Italy</td>
<td>22</td>
<td>Cancer treatment</td>
<td>Oncology ward</td>
<td>2 (bioMérieux, Florence, Italy), 16S rDNA sequencing</td>
<td>CVC (18 patients) Undetermined (4 patients)</td>
<td>Piperacillin/tazobactum</td>
<td>Recovered</td>
</tr>
<tr>
<td>Authors</td>
<td>Country</td>
<td>Gender</td>
<td>Age/Condition</td>
<td>Symptoms</td>
<td>Location</td>
<td>Pathological Test</td>
<td>Antimicrobial Therapy</td>
<td>Outcome</td>
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</tr>
<tr>
<td>Lim et al. 2017</td>
<td>Malaysia</td>
<td>65/F</td>
<td></td>
<td>Fever with chills and rigors</td>
<td>Hemodialysis unit</td>
<td>Not reported</td>
<td>Ceftazidime</td>
<td>Recovered</td>
</tr>
<tr>
<td>Shankar et al. 2018</td>
<td>India</td>
<td>5 cases</td>
<td></td>
<td>Fever with chills, tachycardia, hypertension, fatigue, loss of appetite</td>
<td>Hemodialysis unit</td>
<td>Not given</td>
<td>Sterile water for IV drug preparation</td>
<td>Fluoroquinolones, Cefepime, cefoperazone/ sulbactum</td>
</tr>
<tr>
<td>Souza et al. 2018</td>
<td>Brasil</td>
<td>3 cases</td>
<td>Sepsis</td>
<td>NICU</td>
<td>Vitek 2, 16S rDNA sequencing and PFGE</td>
<td>Not found</td>
<td>Cefepime, Meropenem, Vancomycin</td>
<td>Recovered</td>
</tr>
<tr>
<td>Boattini et al. 2018</td>
<td>Italy</td>
<td>44/M</td>
<td>Fever</td>
<td>ICU</td>
<td>16S rRNA sequencing</td>
<td>CVC</td>
<td>Cefepime25</td>
<td>Recovered</td>
</tr>
<tr>
<td>Basso et al. 2019</td>
<td>Italy</td>
<td>46/F</td>
<td>Fever</td>
<td>ICU</td>
<td>MALDI TOF MS (bioMérieux), 16S rDNA gene sequencing</td>
<td>Not found</td>
<td>Cotrimoxazole, ciprofloxacin</td>
<td>Recovered</td>
</tr>
<tr>
<td>Owusu et al. 2019</td>
<td>Ghana</td>
<td>2/F</td>
<td>Sepsis</td>
<td>OPD</td>
<td>API-20NE (bioMérieux, Florence, Italy), 16S rDNA sequencing</td>
<td>Not given</td>
<td>Cefuroxime</td>
<td>Recovered</td>
</tr>
<tr>
<td>Chitre et al. 2019</td>
<td>India</td>
<td>6 cases</td>
<td>Fever with chills, loss of appetite, generalized weakness</td>
<td>Oncology ward</td>
<td>VITEK 2 system (BioMérieux)</td>
<td>Not found</td>
<td>Piperacillin tazobactum, Levofloxacin</td>
<td>Recovered</td>
</tr>
<tr>
<td>Said et al. 2020</td>
<td>South Africa</td>
<td>16 cases</td>
<td>Sepsis</td>
<td>Hemodialysis center</td>
<td>Vitek 2 (bioMérieux, Florence, Italy), ERIC-PCR</td>
<td>Water in dialysis system</td>
<td>Not reported</td>
<td>15 recovered, 1 died</td>
</tr>
<tr>
<td>Carreira et al. 2020</td>
<td>Portugal</td>
<td>60/M</td>
<td>Infective Endocarditis</td>
<td>Hemodialysis center</td>
<td>Not given</td>
<td>CVC or aortic valve</td>
<td>Cotrimoxazole, Ciprofloxacin (later found resistant)</td>
<td>Died (co-morbidities and co-infections)</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Country</td>
<td>Gender (Age)</td>
<td>Symptoms</td>
<td>Site</td>
<td>Method</td>
<td>MIC</td>
<td>Antimicrobial</td>
<td>Status</td>
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<tr>
<td>Rajendran UD et al. 2021 [21]</td>
<td>India</td>
<td>4 cases</td>
<td>Sepsis</td>
<td>NICU</td>
<td>-</td>
<td>Not found</td>
<td>Fluoroquinolones, cotrimoxazole</td>
<td>Recovered</td>
</tr>
<tr>
<td>Tu J et al. 2021 [22]</td>
<td>China</td>
<td>48/M</td>
<td>Sepsis and multiple organ dysfuntion syndrome</td>
<td>Proctology department</td>
<td>Bruker MALDI-TOF MS</td>
<td>Not found</td>
<td>Levofloxacin, ceftriaxone</td>
<td>Recovered</td>
</tr>
<tr>
<td>Ramani VK, et al. 2021 [10]</td>
<td>India</td>
<td>17 cases</td>
<td>Chemotherap y cycle</td>
<td>Oncology hospital</td>
<td>ViTek 2 Compact system (Biomeriux)</td>
<td>Not found</td>
<td>Cefoperazone sulbactum, ceftazidime, meropenem</td>
<td>Recovered</td>
</tr>
<tr>
<td>Present study</td>
<td>India</td>
<td>38/F</td>
<td>Chemotherap y cycle</td>
<td>Oncology hospital</td>
<td>MALDI-TOF MS</td>
<td>Not found</td>
<td>Imipenem</td>
<td>Recovered</td>
</tr>
</tbody>
</table>


**CONCLUSION**

*R. mannitolilytica* might be more widely distributed than previously thought and targets the immunocompromised and in-vivo device patients. The chemo port was probably inhabited with the strain. Although the source of infection was not sought, correct identification and antimicrobial susceptibility pattern was found essential in the recovery of patient. Active surveillance and multicentric studies to standardise the MICs for *Ralstonia spp.* are therefore recommended.

**REFERENCES**


