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Original Research

Monoclonal Outbreak of *Ralstonia mannitolilytica* Bacteraemia in Oncology Ward

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Abstract

Ralstonia mannitolilytica is a waterborne bacteria found in moist environments. Even though *R. mannitolilytica* is with low virulence, it is found to be a potential pathogen in hospital settings in recent times. The ability of *Ralstonia* spp to survive the commonly used methods of sterilization is the main reason for infections caused by them. Hence, the infections are seen especially in patients with invasive devices such as central venous catheters (CVCs) and are always invasive in nature like bacteremia, pneumonia meningitis, etc. We report an outbreak of *R. mannitolilytica* catheter related blood stream infections (CRBSI) in an oncotherapy ward. A total of 18 confirmed cases were detected using Vitek-2 and MALDI TOF MS. Pulsed field gel electrophoresis (PFGE) was used to determine the clonality of the strains. Antibiotic sensitivity and resistance pattern in *R. mannitolilytica* is also discussed.

***Keywords:** Ralstonia mannitolilytica, Central Venous Catheters (CVCs), Catheter Related Blood Stream Infections (CRBSI), Pulsed field gel electrophoresis (PFGE), Monoclonal Outbreak.*

Introduction

Genus *Ralstonia* was first established in 1995 and was initially classified in the Pseudomonaceae family with one identified pathogen, *Ralstonia pickettii*. Currently *Ralstonia* belongs to Burkholderiaceae family of aerobic, non-fermentative, Gram-negative bacteria (NFGN) with total six recognised species [1]. *Ralstonia* spp are environmental bacteria found in moist environments like soil, water, and plants [2.3]. Genus *Ralstonia* contains some plant pathogens and *Ralstonia pickettii*, *Ralstonia insidiosa* and *Ralstonia mannitolilytica* as opportunistic human pathogens [2,3]. These organisms are usually of low virulence and can survive adverse climates like nutrient deficient surroundings and high temperatures. Their size gives them advantage of passing through 0.2 μm filters that are used to sterilize solutions. These organisms are found in various types of disinfectants and thus conferring the ability of being potential pathogens in hospital settings [4]. Recent studies indicate a rise in infections by *Ralstonia* spp especially in patients with extremes of ages, immunosuppressed and critically ill patients [5,6]. The infections caused are severe invasive infections, including bacteremia (especially central-venous-catheter-related), pneumonia, meningitis, osteomyelitis, etc. [4]. Here, we report a monoclonal outbreak of 18 cases of *R. mannitolilytica* catheter related bloodstream infection (CRBSI) in an oncologic day ward of a tertiary care hospital situated in western India.

Materials and Methods

Hospital settings

This prospective study was conducted at a 50 bedded tertiary care hospital which has one oncology day ward consisting of a single therapy room for chemotherapy infusion and attended by an average of 5 patients /day. This oncology day ward was affected by the outbreak and was the object of the epidemiological investigation. During study duration from March to May 2021, 98 patients attended oncology day ward.

This investigation was conducted by the department of Microbiology assisted by an infection control nurse who reviewed the medical records of the cases to identify any common medical procedure or any occurrence that might have posed a risk for acquiring *Ralstonia* spp. infection.

Epidemiological case definition

A case of CRBSI was defined as a patient attending oncology day ward between March – May 2021 that had a blood culture and/or a central venous catheter (CVC) tip culture positive for *R. mannitolilytica*.

Environmental sampling

Once the outbreak was established, source identification and transmission process were deemed necessary. Environmental sampling started on 24 March 2021, immediately after *Ralstonia* spp. had been isolated from the blood culture of the fourth case. Laboratory technicians collected the samples using commercially available sterile swabs (Hi Media HiClean Swab - w/Polypropylene stick, Sterile gamma irradiated), following existing departmental guidelines. Samples of therapy room included furniture and electronic devices (n=8), in the drug preparation room (n=8) from personal computer, telephone, fax, medicine cabinet, medicine trolley. The in-use liquid soaps (n=5), their dispensers (n=5) and chlorhexidine (n=4) were also collected. Distilled water and sterile water used for injection were also cultured.

Microbiological methods and typing of isolates

All the environmental samples' swabs were cultured in Tryptic Soy Broth, incubated for 48 hours at 37°C, and plated on chocolate agar and blood agar for bacterial growth assessment.

Blood was collected in aerobic and anaerobic Bactec plus bottles for each patient and was processed in automated blood culture system (BD BACTEC FX Instrument, Becton Dickinson, USA). Once the bacterial growth was observed they were cultured in 5% sheep blood agar plates and incubated at 37^0C under 5% CO_2 for 24 hours, according to the laboratory guidelines. Identification of the isolates was obtained by VITEK MS v.3.0 (MALDI TOF bioMérieux, France). Antibiotic susceptibility tests for assessing the minimum inhibitory concentration (MIC) and detecting the presence of extended-spectrum beta-lactamases (ESBLs) and carbapenem resistance were performed for each isolate using the VITEK 2 system (bioMerieux, France), and following the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines.

Typing was done by Pulsed Field Gel Electrophoresis (PFGE). PFGE was performed by embedding bacterial cells in 1% low-melting-point agarose (Bio-Rad Lab, Hercules, CA, USA) and then lysing with lysozyme and proteinase K. This was followed by digesting chromosomal DNA with SpeI (Thermo Scientific-Fermantas Corporation, Vilnius, Lithuania). The resulting fragmented DNA from the various samples were electrophoresed in 1% pulsed field agarose (Bio-Rad Lab, Hercules, CA, USA) using a CHEF-DR III system (Bio-Rad Lab, Nazareth, Belgium) with a pulse time of 5e70 s for 18 h at 14^0C at 6 V cm. The gel was stained with ethidium bromide (5 mg mL1), visualized under UV light, and then photographed using the ChemiDoc MP Imaging System (Bio-Rad Company, United Kingdom). PFGE patterns were analyzed using BioNumerics software, version 7.5 (AppliedMaths, Saint-Matins-Latem, Belgium) [7,8].

Results

A total of 98 patients were included in this study who attended the Oncology ward during the study duration. According to case definition, we identified 18 patients, 13 males and 5 females, age range 30–84 years old (median age 66), attending an oncologic day ward from March – May 2021 (Table 1). In particular, 12 patients had *Ralstonia* spp. positive cultures from both blood and the CVC tip; four patients didn't have blood culture performed, but because of the clinical symptoms their catheters were removed and cultured and were found positive for *Ralstonia* spp. Other two patients had a positive blood culture but the CVC tips were not tested. Analysis of the medical records revealed that the patients had different types of CVC, in particular 16 had a Port, while two had a PICC (percutaneous introduction central catheter). These patients had different types of cancers who underwent different therapeutic protocols and attended the oncology ward on different days. All patients manifested fever/chills as the chief complaint and indicator of BSI (Blood Stream Infection), with no signs of sepsis. The symptoms and laboratory findings did not correlate with infection at any other site in the patients.

First case of CRBSI was detected on March 15, 2021; and *R. mannitolilytica* was isolated in catheter or/and peripheral blood cultures in 18 patients up until May 10, 2021. By 24th March there were 4 cases, and the outbreak evaluation was initiated. Evaluation began with case confirmation followed by source identification.

Specific antibiotic susceptibility for *Ralstonia* spp is not yet developed and hence MICs of *Psedomonas aeruginosa* ATCC27853 were used as standard. Antibiogram pattern of all the patients in our study was found similar based on Vitek 2 (bioMerieux, France), results, as shown in table 2.

Outbreak investigation included all potential sources of the outbreak. Environmental samples were collected ranging from the furniture, electronic devices, medicine trolley, in-use liquid soaps, chlorhexidine. Distilled water, and sterile water used for injection were also cultured. Unused blood culture bottles were treated as control to exclude pseudo-outbreak. Insertion and maintenance care checklists for CVCs and compliance with these checklists were reviewed.

Culture samples which were taken from the fluids administered via catheters did not yield any bacterial growth. However, because of the investigation in the storage area, it was found that there were leaks, air bubbles, and water drops inside the packaging of distilled water bottles. *R. mannitolilytica* was yielded in the cultures obtained from the surface of distilled water bottles. Once the source of outbreak was established all the distilled water bottles were withdrawn immediately from all over hospital.

Table 1: Data of the 18 cases of *R. mannitolilytica*

Patient	Age	Sex	Type of CVC	Date Of Diagnosis	DTP*	Blood Culture	CVC Tip Culture
1	62	M	PICC	15/03/2021	3 Hrs 40 Mins	+	+
2	56	M	Port	18/03/2021	5 Hrs 20 Mins	+	+
3	84	M	Port	20/03/2021	6 Hrs 15 Mins	+	+
4	66	M	Port	23/03/2021	3 Hrs 20 Mins	+	+
5	65	F	Port	27/03/2021	NA	NA	+
6	66	F	PICC	28/03/2021	4 Hrs 50 Mins	+	+
7	73	M	Port	28/03/2021	4 Hrs 30 Mins	+	+
8	65	M	Port	30/03/2021	6 Hrs 20 Mins	+	+
9	67	M	Port	01/04/2021	NA	+	NA
10	56	M	Port	03/04/2021	3 Hrs 30 Mins	+	+
11	30	F	Port	04/04/2021	NA	NA	+
12	62	M	Port	05/04/2021	2 Hrs 50 Mins	+	+
13	65	M	Port	07/04/2021	4 Hrs 15 Mins	+	+
14	72	F	Port	10/04/2021	NA	+	NA
15	77	M	Port	11/04/2021	3 Hrs 20 Mins	+	+
16	67	M	Port	15/04/2021	NA	NA	+
17	80	F	Port	24/04/2021	5 Hrs 30 Mins	+	+
18	63	M	Port	10/05/2021	NA	NA	+

* DTP- Differential time to positivity

Table 2: Antibiogram of isolated *R. mannitolilytica*

Antibiotic	MIC	Interpretation
Amikacin	≥ 64 µg/mL	Resistant
Cefepime	4 µg/mL	Susceptible
Ceftazidime	16 µg/mL	Intermediate susceptible
Cefoperazone/ Sulbactam	≤8 µg/mL	Susceptible
Ciprofloxacin	≥ 4 µg/mL	Resistant
Colistin	≥ 16 µg/mL	Resistant
Gentamicin	≥ 16 µg/mL	Resistant
Imipenem	4 µg/mL	Susceptible
Levofloxacin	≥ 4 µg/mL	Resistant
Piperacillin/Tazobactam	≥128 µg/mL	Resistant
Ticarcillin/Clavulanic acid	≥128 µg/mL	Resistant

Hypothesis for outbreak was infection due to contaminated distilled water used for flushing and/or drug preparation. To validate our hypothesis, a clonal analysis was performed using PFGE. Isolates with identical patterns were considered genotypically indistinguishable, those that differed by one to three bands were considered closely related, those that differed by four to six bands were considered possibly related, and those that differed by more than seven bands were considered unrelated or different [9]. All *R. mannitolilytica* isolates were monoclonal and identical. A strict vigilance was kept for four months since the last case occurred and no new cases were detected. All patients were fully recovered without any CRBSI complications through catheter removal and antibiotic treatment.

Discussion

Infections in healthcare settings are mostly caused by non-fermentative Gram negative bacteria these days. Multiple nosocomial outbreaks by *R. picketti* have been reported worldwide [10]. However, outbreaks by *R. mannitolilytica* are on the rise. There is increased incidence of *Ralstonia* infections in healthcare settings, particularly in vulnerable patients who need continuous IV access, hemodialysis, nebulization etc. *R. mannitolilytica* has been isolated in newborns and in patients with solid cancer, hematological disease, ventriculoatrial draining for hydrocephalus, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and scleroderma [11]. In this study we report an outbreak by *R. mannitolilytica* in oncology ward over the period of 3 months (March- May 2021) where patients suffering from some or other form of malignancy visited for oncotherapy. This study is amongst the initial outbreaks being reported from India. Table 3 shows the outbreaks of *R. mannitolilytica* in recent years in India.

Table 3: Data of recent Indian outbreaks by *R. mannitolilytica*

Reference	Shankar et al [12]	Chitre G et al [13]	Rajendra et al [14]	Ramani et al [11]	Chauhan et al [15]	Present study
Year of study	2018	2019	2021	2021	2022	2022
No. of cases	5	6	4	17	5	18
Hospital setting	Hemodialysis unit	Oncology ward	NICU	Chemotherapy cycle	Hemato- oncology center	Oncology ward
Microbiological Identification	NA	Vitek 2 compact system (bioMérieux)	NA	Vitek 2 compact system (bioMérieux)	Vitek 2 & MALDI TOF MS (bioMérieux)	Vitek 2 & MALDI TOF MS (bioMérieux), PFGE
Outbreak source	Sterile water for IV drug preparation	Not found	Not found	Not found	Multi dose saline bottles	Outer surface of distilled water bottles
Antibiotic therapy	Fluoroquinolones, Cefepime, Cefoperazone sulbactum	Piperacillin tazobactum, Levofloxacin	Fluoroquinolones, cotrimoxazole	Cefoperazone - sulbactum, Ceftazidime, Meropenem.	Levofloxacin, Doxycycline, co-trimoxazole, cefoperazone - sulbactum	Imipenem, Cefepime, Cefoperazone-sulbactum
Outcome	1 death, 4 recovered	Recovered	Recovered	Recovered	1 death, 4 recovered	Recovered

Ralstonia spp are waterborne, found ubiquitously, and can survive any kind of water source. Source of infections in hospital settings is multifactorial usually beginning at manufacturing level since the organisms can contaminate intravenous drugs, saline solutions, distilled water, blood culture bottles, and other solutions used for patient care [10]. This contamination is due to the ability of *Ralstonia* spp to survive in any extreme climatic conditions and also be able to pass through filters (0.45 -0.2mm size); which are the common sterilizing techniques used for sterilizing the solutions used in hospital [4]. Hence, flushing indwelling catheters with these contaminated solutions can lead to BSIs by *R. mannitolytica*. Lucarelli et al had described the *R. mannitolytica* outbreak in their hospital due to flushing of the CVC by contaminated saline solution [19]. The 2005 outbreak in US mentioned the source to be contaminated Vapotherm, the oxygen delivery devices used in the paediatric patients [16]. The microbiological and epidemiological investigation in the present outbreak detected the source of the contamination as outer surface of distilled water bottles. Hence, all the distilled water bottles were removed from all over the hospital and strict hand hygiene protocol was initiated. In spite of removal of contaminated distilled water bottles, the outbreak lasted for 6 more weeks then after. This stipulates the likelihood of patients being exposed to one or more contaminated bottles of distilled water. Few patients developed clinical symptoms immediately, while others likely had CVC colonization by *R. mannitolilytica*. Any subsequent procedure through CVC, chemotherapy or new saline flushing, might have caused detachment and dissemination of *R. mannitolilytica* from CVC, causing fever and chills. This might have happened even after several weeks from CVC colonization, possibly accounting for the protracted duration of the outbreak.

It is conceivable that biofilm formation might have played a role for these strains to allow adherence to CVCs and subsequent dissemination in the host, following the flushing procedures of the device. Boattini et al had indicated the ability of polysaccharide matrix (biofilm) formation by *R. mannitolytica* which helped in adherence to the indwelling catheter with its dissemination during the flushing process by medical solutions [20]. It is important to identify the cause and source of outbreak like we found in present study as it helps curbing the spread of infections and preventing prolonged infections or cause fatal outcomes which *R. mannitolytica* is capable of.

Table 3 clearly shows use of high-level antibiotics and also use of different groups of antibiotics for treatment of *R. mannitolytica* infection. High level antibiotic resistance especially in beta lactams and aminoglycosides is a big challenge in management of these infections. Furthermore, the ability of biofilm formation by *R. mannitolytica* is responsible for added antibiotic resistance mandating testing for antibiogram pattern. Molecular testing was done in certain studies for finding resistant genes like AmpC B-lactamase and oxacillinase. OXA-443 and OXA-444 genes coding AmpC B-lactamase were found by Lucarelli et al and Basso et al in the *R. mannitolytica* strains [19,21]. bla-OXA-22 and bla-OXA-60 coding for narrow spectrum oxacillinase and an inducible carbapenemase, were found by Lucarreli et al [19]. In present study, carbapenems were found susceptible with patients recovering by the treatment. The CVCs were removed in all of our patients which helped in improvement of their condition.

Conclusion

This study highlights that a large number of immunosuppressed patients and rise in invasive procedures have increased the clinical effect and importance of low virulence pathogens such as *R. mannitolilytica.* Accurate identification of the outbreak agent facilitates the identification and control of possible outbreak sources with the evaluation of previous data. Effective active surveillance system and accurate and rapid conduction of microbiological investigations are essential for hospital outbreak management.

Conflict of Interest

The author declares no conflict of interest

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