Original Article

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Microbiology Section

An Outbreak of *Ralstonia mannitolilytica* Septicaemia at a Tertiary Care Hospital: An Observational Cross-sectional Study

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ABSTRACT

Introduction: *Ralstonia* spp. is an emerging non fermenting Gram negative bacillus implicated in cases of bloodstream infections in immunocompromised individuals. It is commonly found as an environmental contaminant in hospital settings. Several sporadic outbreaks have been reported from different parts of the world due to *Ralstonia* spp. This study reports a similar outbreak at a tertiary care hospital in Northern India.

Aim: To determine the source of *Ralstonia* septicaemia in affected patients at a tertiary care centre.

Materials and Methods: The present observational cross-sectional study was conducted at the Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India from February 2020 until the end of March 2020 (two months). A total of 2,650 blood cultures were received during the study period; of these, 53 (2%) patients were found to have *Ralstonia mannitolilytica* infection over a two month period. All patients from various wards whose blood cultures showed growth of *Ralstonia* species were included in this study. The organism was identified using both biochemical tests and Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS). Antibiotic sensitivity testing was conducted using the Kirby-Bauer disk diffusion assay. Environmental surveillance was conducted to detect the source of origin. Patients' age, sex, duration of hospital stay, co-morbidities, and other clinical parameters were recorded. Statistical analysis was performed using Microsoft Excel.

Results: There were 52 cases of septicaemia due to *Ralstonia mannitolilytica*, and one *Ralstonia* isolate was obtained from intraoperative pus. Most of the *Ralstonia* isolates obtained were Multidrug-Resistant (MDR), showing resistance to imipenem, meropenem, amikacin, aztreonam, and sensitivity to first-line drugs such as ceftazidime, piperacillin-tazobactam, cefoperazone-sulbactam, levofloxacin, and trimethoprim-sulfamethoxazole, resulting in successful treatment. Out of 53 cases, one patient succumbed to death due to surgical complications. Environmental sampling did not yield any organisms resembling *Ralstonia* spp.

Conclusion: The environmental source of the *Ralstonia* bacteraemia outbreak could not be identified in this study. All patients except one were successfully treated with antibiotics. Clinicians and microbiologists should remain vigilant in case any such case arises to prevent further outbreaks.

Keywords: Emerging, Multidrug resistance, Non fermenter, Nosocomial, Surveillance

INTRODUCTION

Ralstonia spp. are a group of Non Fermenting Gram Negative Bacilli (NFGNB) that have emerged as a major cause of opportunistic infections in recent decades, particularly among the immunocompromised population. The three important species known to cause disease in humans are *Ralstonia pickettii, Ralstonia insidiosa*, and *Ralstonia mannitolilytica* [1,2]. They are ubiquitous environmental contaminants, commonly found in hospital settings [1-3]. *Ralstonia* spp. have been identified as contaminants in solutions such as sterile water, intravenous medications, skin disinfectants, blood culture bottles, and saline solutions used in hospitals for patient treatment [4,5]. There have been reports of pseudo-outbreaks, where the solutions used for the identification of the organisms were contaminated by these bacteria, leading to false-positive reports [6,7]. These organisms tend to produce biofilms on various intravascular devices and have been implicated in causing various nosocomial infections; they are usually resistant to multiple antibiotics [8]. With the advent of MALDI-TOF MS and other automated identification systems, there has been an increased detection of this organism, which was under-reported in earlier times. Sporadic outbreaks have been reported from different parts of the world [1,2,4,5]. This study reports an outbreak of *Ralstonia mannitolilytica* septicaemia at a tertiary care centre in Northern India that occurred just before the start of the COVID-19 pandemic. The outbreak subsided soon after various wards were closed due to COVID-19. The aim of the study was to determine the source of *Ralstonia* septicaemia in the affected individuals at a tertiary care centre. The clinical characteristics of the patients, the antibiotic treatment given for *Ralstonia* bacteraemia, and the antibiotic susceptibility pattern of the *Ralstonia mannitolilytica* isolates were also studied in detail as objectives of the study.

MATERIALS AND METHODS

This observational cross-sectional study was conducted at the Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, from February 2020 until the end of March 2020 (a period of two months). Out of the 2,650 blood cultures received during the study period, 53 (2%) patients were found to have *Ralstonia mannitolilytica* infection. This was a time-bound study; hence the samples available in the study duration were taken into consideration.

Ethical clearance was obtained from the Institutional Ethics Committee (PGI/BE/224/2020). All patients from whom BACTEC blood culture bottle sets (consisting of one aerobic and one anaerobic bottle) were received in the bacteriology laboratory and tested positive for *Ralstonia* spp. were included in the study. The identification of *Ralstonia* species was performed using colony morphology, biochemical tests such as the oxidase test, and MALDI-TOF MS (Biomerieux, France).

Inclusion criteria: Only the first *Ralstonia* isolate from the blood culture of each patient was included in the study.

Exclusion criteria: Any repeat *Ralstonia* spp. isolates from the blood cultures of the same patients were excluded from the study.

Patients' demographic features and clinical records were obtained from their medical files and entered into a proforma. All medical records were thoroughly analysed to identify any common surgical procedures or interventions that could have posed a risk for *Ralstonia* species infection. The mean age, duration of hospital stay, procalcitonin levels, and Total Leukocyte Counts (TLC) were calculated. The percentages of patients with various co-morbid conditions and the results of antibiotic susceptibility tests were also measured.

Antimicrobial Susceptibility Testing (AST): Manual AST was performed on all *Ralstonia* spp. culture isolates using the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Mueller-Hinton Agar (MHA) was used for susceptibility testing. Antibiotic disks were procured from Himedia Laboratories, Mumbai. The disks used included ceftazidime, levofloxacin/ciprofloxacin, imipenem, meropenem, amikacin, piperacillin-tazobactam, ticarcillin-clavulanate, trimethoprim-sulfamethoxazole, aztreonam, doxycycline, and cefoperazone-sulbactam [1]. Testing for Minimum Inhibitory Concentrations (MICs) was not performed; only the disk diffusion test was conducted. Since there are no CLSI clinical breakpoints for *Ralstonia* species, the results were interpreted according to the CLSI M-100 2019 guidelines for *Pseudomonas* spp. [8,9]. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as control strains for AST.

Surveillance sampling: Infection control nurses collected environmental samples from different wards from which the majority of *Ralstonia* spp. isolates were obtained after five days from the first case. Commercially available sterile swabs were used to collect samples from patients' immediate surroundings, such as bed rails, intravenous sets, and intravenous cannulas. Samples from unused sterile intravenous fluids, liquid soaps, and disinfectants (70% chlorhexidine, alcohol) in use were also collected in sterile universal containers. Drinking water (from reverse osmosis systems) and tap water were collected in sterile containers as well. Dialysis water was obtained from the dialysis unit. Air bioload assessments were conducted using a sieve impactor [10].

Processing of environmental samples: Swabs were incubated in Brain-Heart Infusion (BHI) media at 37°C for 18-24 hours. After 24 hours, the BHI media were visually inspected for any turbidity or growth. A small volume of the sample was then taken with an inoculating loop and subcultured onto blood and MacConkey agar plates. These culture plates were further incubated for 24 hours at 37°C. Any positive growth was subsequently identified using Gram staining and appropriate biochemical tests.

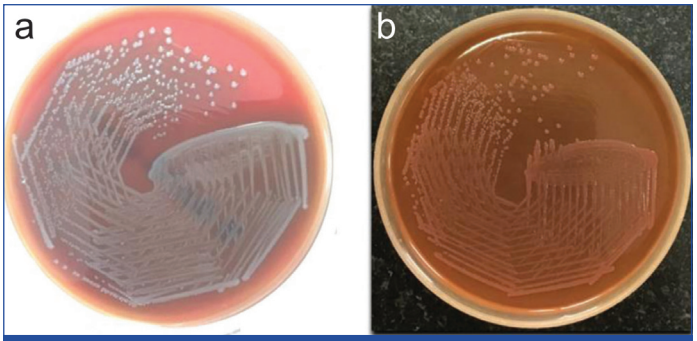
STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft Excel, and the data were analysed using descriptive statistics.

RESULTS

A total of 2,650 blood cultures were received during the study period, of which 53 (2%) patients were found to have *Ralstonia mannitolilytica* infections over a two month period. The colonies of *Ralstonia mannitolilytica* on blood agar were grey, moist, and round to oval, measuring 1-2 mm in diameter. On MacConkey agar, as shown in [Table/Fig-1], there were round to oval, non-lactose fermenting colonies with entire margins, measuring about 2×1 mm. Biochemically, they were catalase positive, oxidase positive, and urease positive. They did not reduce nitrate to nitrite and acidified glucose and lactose but did not produce acid from sucrose. The final identification was performed using MALDI-TOF MS (Biomerieux, France).

One case was identified from intraoperative pus, specifically from a splenic abscess. The remaining 52 strains were isolated from BACTEC blood cultures. Of the 53 patients, 33 (62.26%) were males and 20 (37.74%) were females. All patients presented with



[Table/Fig-1]: Colonies of *Ralstonia mannitolilytica* on (a) Blood Agar and (b) MacConkey agar.

febrile episodes during their admission. The highest number of cases originated from the Nephrology, Emergency, and Surgical Gastroenterology (SGE) wards. The distribution of cases from different wards is shown in [Table/Fig-2].

Ward	No. of patients (%)
Nephrology	18 (34)
Emergency	15 (28)
Surgical gastroenterology	10 (19)
Haematology	2 (3.8)
Cardiology	1 (1.9)
Endocrinology	1 (1.9)
Radiotherapy	1 (1.9)
Pulmonary medicine	1 (1.9)
Immunology	2 (3.8)
Paediatrics	2 (3.8)

[Table/Fig-2]: Ward-wise distribution.

The medical records of all patients were thoroughly investigated. A total of 33 patients (62.26%) were found to have different types of Central Venous Catheters (CVCs). [Table/Fig-3] presents the various types of intravenous catheters found in 53 patients with *Ralstonia mannitolilytica* septicaemia. Fourteen patients (26.4%), who were receiving haemodialysis, had both Arteriovenous (AV) fistula and peripheral venous catheters.

Type of intravascular catheter	N (%)
Central Venous Catheter (CVC)	24 (43)
Peripherally inserted Central Venous Catheter (PICC) line	09 (17)
Arteriovenous fistula for haemodialysis and peripheral venous catheter	14 (26.4)
Peripheral venous catheter	06 (11.3)

[Table/Fig-3]: Types of intravascular catheters.

The clinical details of the patients are shown in [Table/Fig-4]. The mean age of the patients was 47.17 years. The most common co-morbidities found were diabetes and hypertension. The procalcitonin and TLC levels were found to be elevated in most of the patients.

Demographic and clinical parameters	Values
Average age (years)	47.17 (Minimum age-3 years; Maximum age- 93 years)
Average duration of total hospital stay (days)	20.7
Average duration of hospital stay before positive blood culture (days)	9.3
Mean procalcitonin levels ng/mL (normal range-<0.25 ng/L)	6.98 ng/mL
Mean TLC levels cells/ mm^3 (normal range-4000-11000/mm^3)	14,612
Co-morbidities	**N (%)**
Diabetes	16 (30.2)
Hypertension	17 (32)

CKD	12 (22.6)
Postrenal transplant	4 (7.5)
COPD	4 (7.5)
Bronchial asthma	3 (5.7)
Liver disease	6 (11.3)
Haematological malignancy	6 (11.3)
Solid organ malignancy	4 (7.5)
Tuberculosis	3 (5.7)
Others (hepatitis B, Cushing disease, Hypothyroidism, Rheumatoid arthritis)	6 (11.3)

[Table/Fig-4]: Demographic and clinical parameters of patients with *Ralstonia* cases during the current study period.
CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease

Empirical antibiotics were initiated for all patients based on their clinical presentations and were de-escalated following the culture and antibiotic susceptibility reports. Antibiotic susceptibility testing revealed that all isolates were resistant to amikacin, meropenem, imipenem, ticarcillin-clavulanate, and aztreonam. The most effective drugs were cefoperazone-sulbactam and levofloxacin. The antibiotic susceptibility pattern of all 53 isolates is shown in [Table/Fig-5].

Antibiotics	Sensitive N (%)	Resistant N (%)
Cefoperazone-Sulbactam	53 (100)	0
Levofloxacin	53 (100)	0
Trimethoprim-sulfamethoxazole	50 (94)	3 (6)
Ceftazidime	37 (70)	16 (30)
Piperacillin-tazobactam	32 (60)	21 (40)
Doxycycline	1 (2)	52 (98)
Meropenem	0	53 (100)
Imipenem	0	53 (100)
Amikacin	0	53 (100)
Ticarcillin-clavulanate	0	53 (100)
Aztreonam	0	53 (100)

[Table/Fig-5]: Antibiotic susceptibility patterns of *Ralstonia* isolates obtained during the current outbreak.

Out of 53 patients, one patient died due to complications during surgery, while the remaining 52 patients recovered with appropriate antibiotic therapy. The mean time to the first subsequent negative blood culture was 11 days in these patients. Environmental sampling was conducted at various hospital sites. All environmental samples were found to be sterile. None of the surveillance samples grew *Ralstonia* spp. Therefore, the source of the outbreak could not be detected.

DISCUSSION

During the current outbreak, 52 cases of *Ralstonia mannitolilytica* bacteraemia were observed, along with one case from intraoperative pus, specifically from a splenic abscess. Prior to this outbreak, the same institution reported only a few cases of *Ralstonia*, primarily in immunocompromised patients with conditions such as haematological malignancies, etc. However, the sudden increase in the number of *Ralstonia* cases has prompted urgent efforts to identify the source of the outbreak. The first case report of *Ralstonia* spp. in India was from this institute, involving a 14-year-old renal transplant recipient in 2003. The child was saved through prompt microbiological identification and appropriate antibiotic treatment [11]. Subsequently, various isolated cases have been reported from India and globally [11-14]. Liu CX et al., reported three cases of *Ralstonia mannitolilytica*-induced septicaemia in 2016; all were postoperative, and the environmental source of origin was not identified [13].

Until the last decade, the most commonly isolated species from clinical specimens in reported cases was *Ralstonia pickettii*, but more recently, cases of *Ralstonia mannitolilytica* as well as *Ralstonia insidiosa* have emerged [15]. In this study, all cases were due to *Ralstonia mannitolilytica*. Rajendran UD et al., recently reported an outbreak of *Ralstonia mannitolilytica* sepsis in a neonatal Intensive Care Unit (ICU) [16].

Ralstonia has been identified as a potential contaminant in various skin disinfectants, distilled water used in respiratory equipment, intravenous medications, blood culture bottles, heparinised syringes, and saline solutions in the hospital setting, due to its ability to grow in a wide range of temperatures (15°C to 42°C) [17-19]. In a study by Lucarelli C et al., the replacement of multidose saline infusions used for CVC flushing with single-dose vials led to the control of a *Ralstonia* sepsis outbreak [20]. The increased use of indwelling blood catheters and the organism's propensity to produce biofilms have also contributed to a rise in infections by these less virulent pathogens [15]. In this study, 33 (62.26%) patients had CVCs, and 14 (26.4%) patients had AV fistula for haemodialysis.

Ralstonia species are frequently resistant to various commonly used antibiotics, including β-lactams and most aminoglycosides [21]. In this study, all isolates were resistant to carbapenems (imipenem and meropenem), amikacin, and ticarcillin-clavulanate. Empirical antibiotics were initiated for all patients based on their clinical presentation and subsequently de-escalated after receiving culture and antibiotic susceptibility reports. In this case, de-escalation to cefoperazone-sulbactam and levofloxacin was recommended to clinicians, as all *Ralstonia* isolates were sensitive to these drugs. Similarly, Ryan MP and Adley CC recommended quinolones and trimethoprim-sulfamethoxazole for the treatment of *Ralstonia* species [21]. In this study, 3 (6%) isolates were resistant to trimethoprim-sulfamethoxazole. All patients survived except one, who died due to multiple surgical complications.

Environmental sampling was carried out at various sites within the hospital. However, all cultures were found to be sterile. The outbreak, which spread across various wards in the same building and involved patients presenting on different days upon admission, made it difficult to identify the source. Other authors have reported *Ralstonia* spp. outbreaks where the environmental source remained undetected [1,20]. The outbreak resolved on its own when patient admissions were halted due to the lockdown during the first COVID-19 pandemic. Hand hygiene, social distancing, and stringent sanitisation measures, adopted in response to COVID-19 cases, were major factors in the self-containment of the outbreak. This was not a pseudo-outbreak, as samples were collected from different wards/OPDs by different personnel. Additionally, sets of blood cultures were sent for each sample, and the same lot of blood culture bottles was used for other patients who had negative culture results during the same period.

Limitation(s)

Due to the onset of the COVID-19 pandemic, detailed environmental sample surveillance could not be conducted across all wards. MICs were not tested, and epidemiological typing was not performed for the isolates due to financial constraints.

CONCLUSION(S)

Ralstonia mannitolilytica is an emerging nosocomial pathogen with the ability to cause outbreaks in immunocompromised populations. The environmental source of origin could not be detected in this study. All patients, except one, were successfully treated with levofloxacin and cefoperazone-sulbactam. Keen suspicion by clinicians and microbiologists is required to take prompt action to prevent such outbreaks by removing environmental sources or contaminated medical solutions/devices.

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