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Nosocomial bloodstream infection and the emerging carbapenem resistant pathogen *Ralstonia mannitolilytica*: A case series

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Abstract

Ralstonia mannitolilytica (RM) is a rare opportunistic pathogen capable of causing a serious infection in immunocompromised patients, especially oncology patients with prolonged presence of indwelling central catheters. Gram negative non fermenting bacteria are an emerging concern in clinical locations being a common cause of nosocomial infections. In this study, we report a case series of RM infection with focus on common clinical characteristics and patterns of their antibiotic sensitivity and resistance pattern with specific stress on Carbapenem resistance. The case series includes 12 patients admitted at Vydehi Institute of medical college and research centre Bangalore, presenting with fever, chills and other signs of infection between 4th October 2020 to 21nd February 2021 who had positive blood culture and PICC/central venous catheter (CVC) tip culture positive for RM species. Out of 12 patients RM was grown in blood sample of 7 patients with central venous catheter, 3 with indwelling chemo Port, 2 with PICC line. All the patient's blood culture was positive for RM with same resistance pattern. In conclusion our study shows that RM is a new emerging carbapenem resistant gram-negative organism which can be life threatening especially in immunocompromised patients. Its incidence must immediately warrant an active search for source of contamination. This could be an indirect indicator of quality of water reservoir and other supplies used in these patients. The sensitivity pattern of RM being resistant to many routinely used antibiotics, even carbapenems. The case series serves an alert for medical workers and researchers to pay more attention to infection caused by RM.

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Introduction

There is increased susceptibility for blood stream infections among cancer and other immunocom promised patients due to underlying malignancy, malnutrition, immunosuppression steroids which all contribute to increased mortality and morbidity (Ramani *et al*., 2021a). Central venous catheter whether implanted or non-implanted are frequently used for administration of therapy among patients with malignancies. CVC can be a source for central line associated bloodstream infections (CLABSI) (Ramani *et al*., 2021a).

Gram negative non fermenting bacteria are an emerging concern in clinical locations being a common cause of nosocomial infections (Ryan and Pembroke 2018). The genus *Ralstonia* comprises a group of non-fermentative, Gram-negative bacteria (NFGN) found in moist environments, such as water, soil and plants (Lucarelli, Di Domenico, *et al*., 2017). Three *Ralstonia* species, *Ralstonia Pickettii, Ralstonia insidiosa* and *Ralstonia mannitolilytica* (Lucarelli, Domenico, *et al*., 2017). Formerly designated *Burkholderia pickettii, Burkholderia solanacearum* and P*seudomonas thomasii, respectively Ralstonia pickettii* is a slow growing and produces only pinpoint colonies on blood agar after 24 hours.

All the strains are urease positive and some strains may be catalase negative. Motility is weak or delayed and may not be detectable (Koneman *et al*., n.d.). *R. mannitolilytica* is name given to organism formerly classified as *R. picketti* biovar 3/'thomassi. It can be distinguished from all described *Ralstonia* species by acidification of D-arabitol and mannitol and its lack of nitrate reduction and of alkalization of tartrate (Koneman *et al*., n.d.). *R. pickettii* is a slow growing and produces only pinpoint colonies on blood agar afterhours. All the strains are urease positive and some strains may be catalase negative.

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Their relevance has been currently re-evaluated because of their ability to survive in different types of disinfectants and to passthrough 0.2-µm filters that are used to sterilize solutions *Ralstonia* spp. is reported as causative agent of bacteremia, meningitis and sepsis in immunocom promised patients and of central venous catheter (CVC)-associated bacteremia in oncology patients (Lucarelli, Domenico, *et al.,* 2017). Several hospital outbreaks have been described that were associated with contaminated solutions, including water for injection, saline solutions, disinfectants and antiseptics Multidrug resistance in NFGN (non-fermenting gram negative bacilli) 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. Several studies have described resistance to fluoroquinolones, 3rd generation cephalosporin and carbapenems in isolates belonging to all the three *Ralstonia* species. *R. pickettii* is the *Ralstonia* species most frequently reported in the literature while only a limited number of infections are attributed to *R. insidiosa* and *R. mannitolilytica* (Fang *et al*., 2019).

The clinical importance of these two species is probably underestimated because their biochemical patterns are similar to that of *R. pickettii,* making it impossible their distinction based on conventional microbiological tests only (Fang *et al*., 2019).

Ralstonia spp, are emerging as global opportunistic pathogens affecting immunocom promised patients.

Even without person to person transmission, the prevalence of *Ralstonia* infection is notably increasing. The bacteria reproduce in wet conditions and can survive in harsh environments (even in weak disinfectants) for long periods. The species exist widely in external aqueous environments including municipal water and medical water purification systems (Lucarelli, Domenico, *et al*., 2017).

As the bacteria can pass through 0.2-μm filters during the sterilization process, medical products may be contaminated during the manufacturing phase (Fang *et al*., 2019).

The species can create biofilms on the surfaces of medical supplies and produce toxins. Many infectious cases caused by *R. picketti* and *R. mannitolilytica* are due to the use of contaminated solutions, blood product chlorhexidine, saline solution, and sterile water as well as the colonization of medical devices (tap water and water used for hemodialysis, bronchoscope flushing, and heparin for flushing) (Fang *et al*., 2019). To assess *Ralstonia mannitolilytica* as an emerging new bacterial strain as a causative agent for nosocomial infection in immunocom promised patients and to see the resistant pattern among the isolated strains.

Materials and methods

This case series included 12 patients and their clinical information was analyzed. The cases include patients admitted in oncology unit and neonatal ICU of Vydehi Institute of Medical Sciences and Research Centre from 4th august 2020 to 22nd February 2021. Patients were admitted for the treatment of different malignancies in oncology ward and in neonatology ICUs for preterm condition, when they presented with fever chills for which blood samples were send for culture and sensitivity. Cultures were positive for *Ralstonia mannitolilytica* and all 12 isolates showed similar sensitivity pattern. The authors reviewed the medical records of all cases identifying any common medical procedure or factors which might have caused the risk of acquiring *Ralstonia* infection. The similarities among all patients were immunosuppressed with either chemo port/PICC or central venous catheter 10 out of 12 cases were from same floor and same ward (oncology ward) of the hospital. Blood culture samples from the peripheral lined along with port/PICC line were sent for microbiological processing.

The demographic characteristics of the patients were also noted.

Environmental Surveillance samples were collected from RO water, hand rub, floor, bed, common sink, biomedical area, heparin injection sterile water and cleaning solutions.

Isolation and Identification

Blood cultures were taken from both the peripheral vein and chemo port or CVC line. These were tested by on Biomerieux automated (Bact /alert 3D) culture system. After growth was detected, they were plated on MacConkey and Sheep Blood (5-7%) agar plates. Subsequently it was incubated aerobically at 37+/-1 degree Celsius for 24-48 hrs. Colonies were then put on VITEK 2 compact system (Biomerieux) using VITEK 2 GN card along with ID card. Next day after 24 hours VITEK was read and result compilated. All the isolates were further again verified by MALDI-TOF (Matrix -assisted Laser Desorption/Ionization-Time of Flight) and were identified to be *Ralstonia mannitolilytica*.

Results

A total of 12 cases were reported over a period of six months. 10 of 12 were from oncology ward and 2 from neonatal ICUs. All cases presented with fever after 48 hours of admission as per the in-patient records patients belonged to different residential areas. There was no growth of *Ralstonia* in any of the surveillance samples. The complete Baseline information/characteristics of 10 patients from oncology are listed in Table 1.

Table 1. Showing baseline information of 10 patients.

Patient	Age/Sex	Comorbidity	Type of IV Line	Type of Cancer
1	7/M	Hypothyroid	PICC	Wilms Tumor
2	45/M	Hypertension	Port	Diffuse large B cell lymphoma
3	68/M	DM	Port	CA Colon
4	75/M	HTN/Type 2 DM	Central	CA Stomach
5	39/M	Hypothyroid	PICC	Papillary CA thyroid
6	79/M	HTN/DM	Central	CA Esophagus
7	69/M	HTN/DM	Central	CA Liver
8	72/M	HTN/DM	Port	CA Stomach
9	58/M	HTN	Central	CA Colon
10	64/M	HTN	Central	CA Liver

HTN: hypertension, DM: Diabetes Mellitus & CA: Carcinoma.

Table 2. Showing baseline information of two neonates in neonatal ICU.

Patient	Age/Gender	Comorbidity	Type of IV Line	Birth Weight
11	30 Weeks	Preterm/LBW	Central	840gms
12	32 Weeks	Preterm/LBW	Central	1200gms

LBW: Low Birth Weight.

Table 3. Shows the antibiotic sensitivity pattern of all 12 isolates of Ralstonia Mannitolilytica.

Antibiotic	Sensitivity (Microgram)	Antibiotic	Sensitivity (MIC)
Ticarcillin/clavulanic acid	Resistant.>=128	Imipenem	Intermediate 8
Piperacillin/tazobactam	Resistant> =128	Meropenem	Resistant>= 16
Ceftazidime	Intermediate 16	Amikacin	Resistant> =64
Cefoperazone/sulbact am	Sensitive <=8	Gentamicin	Resistant>= 16
Cefepime	Sensitive 2	Ciprofloxacin	Sensitive < =0.25
Aztreonam	Resistant. > = 64	Levofloxacin	Sensitive 0.25
Minocycline	Sensitive < =1	Tigecycline	Sensitive <=0.5
Tigecycline	Sensitive <=0.5	Colistin	Resistant> =16
		Trimethoprim/sulfa me thoxazole	Sensitive <=20

Table 4. Treatment received and outcome of patients.

Patient	Treatment	Procedure done	Outcome
1	Cefoperazone/ sulbactam	Changed PICC	Recovered
2	Cefoperazone/sulbactam	Removed port	Recovered
3	Cefoperazone/sulbactam	Removed port	Recovered
4	Cefoperazone/sulbactam	Changed central line	Recovered
5	Cefoperazone/sulbactam	Changed PICC	Recovered
6	Cefoperazone/sulbactam	Changed central line	Recovered
7	Cefoperazone/sulbactam	Changed central line	Recovered
8	Cefoperazone/sulbactam	Changed port	Expired
9	Cefoperazone/sulbactam	Changed central line	Recovered
10	Cefoperazone/sulbactam	Changed central line	Expired
11	Cefoperazone/sulbactam	Changed central line	Expired
12	Cefoperazone/sulbactam	Changed central line	Recovered

The complete baseline information of 2 neonatal cases is depicted in Table 2 Among samples RM was grown from the blood culture of 7 (58%) patients having Central venous line.3(25%) patients with chemoport 2 (16%) patients with PICC line 3 (25%) patients had no co morbidities, while 9 (75%) had comorbidities (hypertension, Type 2 diabetes, and hypothyroid). All the patients had positive blood culture for RM with carbapenem resistance for all isolates. Table 3 shows All the 12 isolates were showing same sensitivity pattern with all sensitive to (Cefoperazone/salbactum, cefepime, minocycline, tigecycline, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole) Resistant to (ticarcillin/clavulanic acid, piperacillin/azobactam, aztreonam, meropenem, imipenem, amikacin gentamicin).

Discussion

Ralstonia are waterborne bacilli implicated in hospital acquired infections. It is a non-pathogenic environmental microbe and clinical infection with this species is rare. However, with the development of modern-day pharmaceuticals, large use of immunosuppressive drugs like steroids, biological therapy etc. in oncology settings and rampant use of broad-spectrum antibiotics has resulted in increased rates of this infection (Ramani *et al*., 2021b). RM has been implicated in few clinical conditions often resulting in subsequent severe meningitis, septicemia and peritonitis (Liu *et al*., 2016).

RM has the ability to pass through 0.2 µm filters which are used for sterilization of many medical products, such as saline solution. This ability enables it to potentially pass through the dialysis reverse osmosis membrane or even a dialyzer. Chlorhexidine with 0.05% aqueous solution is used as a topical antiseptic in venous catheter related procedures (Lim and Lee 2017). Dotis J *et al* report that RM can form a biofilm on plastic catheters (Dotis *et al*., 2012). Basso M *et al* report that members of the genera *Ralstonia* are the significant taxa identified in atherosclerotic plaques removed during surgery (Basso *et al*., n.d.). Regardless of the source of infection, a combination of arterial thrombosis and biofilm related chronic contamination could be the key factors for the persistence and relapse of the Ralstonia species bacteremia, which occurred despite an appropriate and long-term antibiotic therapy (Basso *et al*., n.d.).

There is limited evidence regarding serious non-outbreak related RM infections, but it is essential not to misidentify RM as *Pseudomonas fluorescens, Burkholderia multivorans* and/or *Ralstonia pickettii*, which are often treated as contaminants (Basso *et al*., n.d.). Similar to our study, Mukhopadhyay *et al* study also reports no growth of RM from environmental samples (Mukhopadhyay, 2003 n.d.).

Their study reports that RM was unlikely to be a contaminant from saline solution or deionized water used for parenteral fluids as three consecutive isolates had the same antibiotic sensitivity patern (Mukhopadhyay, 2003 n.d.).

In our case series as well, RM could be not detected either through microbiological or epidemiological investigations. From an epidemiologist and health worker point of view, the possible source of contamination of the RM outbreak in our study was contaminated saline or pharmaceutical supply used in the recent past (Lucarelli, Di Domenico, *et al*., 2017). In their Italian study, report the first outbreak due to RM in oncology patients bearing central venous catheter (CVC). Although a definitive source of the outbreak was not identified, the investigation suggested that contaminated saline solution used for CVC flushing may have been the source. In our study, it was interesting to note that majority of the infections occurred during the period of COVID-19 lockdown where strict hand hygiene practices and social distancing were implemented. As CVC flushing with saline is a common procedure adopted among all oncology patients, it is likely that our study subjects were exposed to one or more contaminated bottles of saline. In our study, a similar batch of sterile water which was earlier used for infusion of the port or peripheral line did not show growth of RM. We could not retrieve the ampoules of sterile water used during the time of outbreak. Lim's study reports that despite the low virulence of RM, it is able to survive in harsh conditions. This could be potentially harmful to many immunocompromised patients (Lucarelli, Di Domenico, *et al*., 2017).

The study focus was on RM infection among dialysis patients, which had occurred during the crisis of municipal reservoir water contamination at Serdang, Malaysia. The authors opine that RM infections at hospital settings are typically associated with contaminated medical supplies or instruments.

In the hospital setting, it has been found that human RM cases are a result of contaminated solutions such as distilled water, injectable water or saline, or purified respiratory ampoules (Lucarelli, Di Domenico, *et al*., 2017). The source of RM related hospital outbreaks could also include contamination of parenteral fluids with deionized water.

The microbiological and epidemiological investigation of this outbreak did not detect the source of the contamination; however, the molecular typing of the pathogen strongly supports the hypothesis of a common source of contamination. Failure to identify the culprit is probably due to the fact that when the investigation was started samples from disinfectants, antiseptics and saline solutions used at the beginning of the outbreak were not available for microbiological investigation. In fact, disinfectants, antiseptics and multi-dose bottles of saline solution have been described in the literature as one of the main sources for *Ralstonia* spp. contamination (Lucarelli, Domenico, *et al*., 2017).

Conclusion

In conclusion our study indicates that though RM infection was not life threatening in most of the cases but its incidence must be taken seriously and should warrant an active search for the search of the source of infection. This could be an indirect evidence about the quality of water and other pharmaceutical supply. In order to prevent such outbreaks and to prevent its emergence as a new nosocomial infection causing pathogen with carbapenem resistance it is mandatory that strict infection control measures be practiced in wards and ICUs especially when dealing with CVC lines of immunocom promised patients. All parenteral treatment for immunocom promised patients with CVC or PTCC to be done under sterile precautions using single dose solutions and mandate the removal CVC. It is the healthcare workers who need to strictly implement infection control policies. Any such outbreak needs to be brought to the notice of HICC so that proper surveillance can be carried. This involves team work of treating doctor's microbiologist and other associated staff.

In this case series the fact that RM is an emerging carbapenem resistant pathogen with potential of causing Nosocomial infection in immunocompromised patients is evident and clear. Hence it is very important on the part of microbiologist to improve the identification of the RM because it is not uncommon practice to misidentify it with Burkholderia and to be considered as contaminant. Although RM is not recognized as a major pathogen, we should be careful as its ability to survive in the environment, it's a potential for emerging as a multidrug resistance and ability to form biofilms.

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