Infection and Drug Resistance

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CASE SERIES

Bloodstream Infections Caused by Drug Resistant *Ralstonia species*: A Case Series During the COVID-19 Pandemic

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Abstract: *Ralstonia spp*. is an emerging, non-fermentative Gram-negative rod that demonstrates multidrug resistance. Herein, four cases of bloodstream infections (BSI) caused by *R. mannitolilytica or R. pickettii* are presented. All the cases had comorbidities that predisposed them to this opportunistic infection. The microbiological assessment showed carbapenemase genes carried out in two strains with minimal inhibitory concentrations > 32 µg/mL to imipenem and meropenem. Fluoroquinolones and trimethoprim-sulphamethoxazole were the most potent agents showing activity against 3/4 strains (75%), although treatment should be susceptibility-dependent for each strain. This case series highlights the possibility of co-infection by a rare organism during the COVID-19 pandemic and the importance of the readiness of diagnostic laboratories to support the diagnosis of uncommon pathogens.

Keywords: multidrug-resistance, bacteremia, bacillary, OXA-48, carbapenemase

Introduction

Despite the availability of effective antimicrobial agents and advancements in supportive care, bloodstream infection (BSI) remains a significant cause of morbidity and mortality.[1] Timely identification and antimicrobial susceptibility testing of the implicated pathogens are essential to implement early effective therapy and improve outcomes. Bacteremia due to Gram-negative bacilli is a significant challenge in both nosocomial and outpatient settings. These pathogens represent serious threats because of the increasing multidrug resistance.[2] The mortality of Gram-negative bacillary bacteremia is estimated to be between 12–38% depending on various factors of which the administration of timely, effective therapy remains crucial.[3,4] Non-fermenters, such as *Ralstonia, Pandoraea, Cupriavidus, Inquilinus, Comamonas, Acidovorax, Delftia, Hyrodenophaga, Brevundimonas, Xanthomonas* and *Achromobacter* are increasingly recovered from refractory bacteremia cases with limited evidence about their optimal treatment.[5] Differentiating these closely related pathogens requires the use of reliable diagnostics such as those based on the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) technology.[6]

There are currently five species of which three are known to cause infections in humans namely *Ralstonia pickettii, Ralstonia mannitolilytica*, and *Ralstonia insidiosa*. Other species that used to be within this genus have been reclassified to the genus *Cupriavidus*. This taxonomic update may hinder accurate laboratory identification. The prevalence of BSIs by *Ralstonia* is unknown as the cases are extremely rare, although the infection by the nosocomial pathogen can occur sporadically or in outbreaks particularly in hemodialysis units.[7,8] Rapid and accurate diagnosis is required especially for invasive infections to implement effective therapy. In this case series, four hospitalized patients diagnosed with *Ralstonia* BSIs are presented from our institution during the COVID-19 pandemic (July 2020–December 2022), along with their clinical outcomes (Table 1). The identification process for all the described strains of *Ralstonia* was performed using the VITEK® MS (bioMe´rieux Inc., Durham, NC, USA), an automated mass spectrometry microbial

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Table 1 Clinical Characteristics of 4 Patients with *Ralstonia* Bloodstream Infections

	Case 1	**Case 2**	**Case 3**	**Case 4**
Year of presentation	2020	2020	2021	2022
Age (Year)	79	55	48	19
Gender	Male	Female	Male	Male
Ethnic group	Arab	Arab	Asian	Arab
Time after hospitalization (d)	22	15	35	26
Primary diagnosis	Pulmonary hypertension	COVID-19 pneumonia	COVID-19 pneumonia	Post-meningitis hydrocephalus
COVID-19 status	Negative	Positive	Positive	Negative
Comorbidities				
Diabetes mellitus	Yes	Yes	No	No
Hypertension	Yes	No	No	No
Dyslipidemia	Yes	No	No	No
Other diseases and risk factors	Osteoporosis, hypothyroidism Central line	Sickle cell disease Post-GI surgery	Central line	Cochlear sclerosis Ventriculoperitoneal shunt
End stage renal disease	No	Yes CKD IV	Yes	No
Immunosuppressive medications	-	-	-	Prednisolone 5 mg daily
Treatment given	Levofloxacin trimethoprim-sulfamethoxazole	Piperacillin-tazobactam	Colistin trimethoprim-sulfamethoxazole	Meropenem
Outcome	Recovered	Recovered	Died	Recovered

identification system based on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology. Antimicrobial susceptibility profiles were obtained by antibiotic disc diffusion assays and upon detection of carbapenem resistance, the strain was tested by E-test (AB-BIODISK, Sweden)-based minimal inhibitory concentrations for both imipenem and meropenem and the production of carbapenemases using a molecular assay (Xpert® Carba-R, Cepheid Inc., Sunnyvale, CA, USA) which detects five major genes (IMP, KPC, NDM, VIM, and OXA-48).

Case Series

The first case was a 78-year-old Saudi male patient with osteoporosis, hypothyroidism who presented with pulmonary hypertension and then a central line was inserted for providing continuously infused therapies. The patient also had history of diabetes mellitus, hypertension and dyslipidemia. He developed fever 22 days after admission, when a slowly growing Gram-negative rod (> 72 hours) was grown from his blood cultures and was identified as *Ralstonia mannitolilytica*. The isolate exhibited a class D carbapenemase, OXA-48, using the Cepheid Genexpert carba-r kit. Levofloxacin was initiated that was then shifted to trimethoprim-sulfamethoxazole, and the patient recovered over a 2-week period.

A second case in the series relates to a 55-year-old Saudi lady with a background of diabetic nephropathy (Chronic Kidney Disease IV), sickle cell disease who presented with COVID-19 pneumonia and a previous gastrointestinal surgery, cholecystectomy, 1 month prior to presentation. Blood culture sampling obtained 15 days after admission revealed a culture growth of *Ralstonia mannitolilytica* visible in 2 days. Despite her co-morbidities, the patient did not

complain of any complications following an empirical meropenem treatment (MIC = 2 µg/mL) that was shifted to piperacillin-tazobactam based on in vitro susceptibility.

The third case was a 48-year-old Asian male patient with an unknown prior morbidities who was admitted as a case of COVID-19 based on radiological and clinical findings and a laboratory PCR confirmation. The patient presented acutely with respiratory failure and required a central line to be inserted during his hospital course in the intensive care unit. He developed bacteremia and sepsis on day 35 of admission, when *Ralstonia pickettii* was isolated with an OXA-48 genes for carbapenemases detected in the isolate. His blood cultures concurrently grew *Acinetobacter baumannii* that was extensively drug resistant. He received an initial dose of meropenem and colistin that was shifted to trimethoprim-sulfamethoxazole and colistin based on microbiology reports. The bacteremia was refractory and the patient passed away.

The last case was a 19-year-old male patient presenting with a malfunctioning ventriculoperitoneal shunt inserted at the age of 7 years to manage post-meningitis hydrocephalus. The patient also has cochlear otosclerosis and was on prednisolone 5 mg daily. The cerebrospinal fluid culture initially grew *Corynebacterium spp*., a Gram-positive low virulence organism that is linked to device infections. The patient was commenced on meropenem based on in vitro susceptibility result and cleared the infection. However, the patient required neurosurgical interventions for his primary illness. He remained clinically stable throughout this episode and no recurrence of BSI was recorded.

Discussion

Ralstonia BSIs are uncommon but are increasingly reported. The recent literature suggests an emerging role of *Ralstonia* species in opportunistic infections, especially in immunosuppressed patients.[9] *Ralstonia* has been linked to a variety of infections including sepsis, infective endocarditis, osteoarticular infections, and severe pneumonia.[9] Our institution encountered four cases of BSI during the pandemic; two of which were isolated from confirmed COVID-19 cases (Table 1). It was recently observed that *Ralstonia* represents an abundant component of the microbiome of SARS-CoV2 infected patient compared to their counterparts, suggesting a possible effect of COVID-19 in predisposing to *Ralstonia* infections.[10] Further studies are needed to elaborate the role of the inflammation imposed by the novel virus in favoring growth of infrequent low-virulence pathogen.

Ralstonia spp. grows well on commonly used laboratory media including blood and MacConkey agars, but growth may require >72 h of incubation to be well visualized (Table 2, Figure 1). Due to its biochemical profile, *Ralstonia* spp. can be misidentified in the laboratory as *Burkholderia cepacia* complex.[10] Reliable identification to a specie level requires techniques that include MALDI-TOF, 16S ribosomal RNA (16S rRNA) and other nucleic acid amplification assays which are not standard routine tests for many microbiology laboratories.[6,11,12] Nevertheless, MALDI-TOF based

Table 2 Laboratory Features of 4 *Ralstonia* Spp. Isolated from Bloodstream Infections

	Culture Growth	Biochemical Profile	Molecular Testing for Carbapenemases	MIC for Carbapenems µg/mL	
				Imipenem	Meropenem
Strain 1 *Ralstonia mannitolilytica*	72 hours Non-lactose fermenter	Oxidase + Catalase +	OXA-48	>32	>32
Strain 2 *Ralstonia mannitolilytica*	48 hours Non-lactose fermenter	Oxidase + Catalase +	-	3	2
Strain 3 *Ralstonia pickettii*	72 hours Non-lactose fermenter	Oxidase + Catalase -	OXA-48	>32	>32
Strain 4 *Ralstonia mannitolilytica*	48 hours Non-lactose fermenter	Weak oxidase + Catalase +	-	4	4

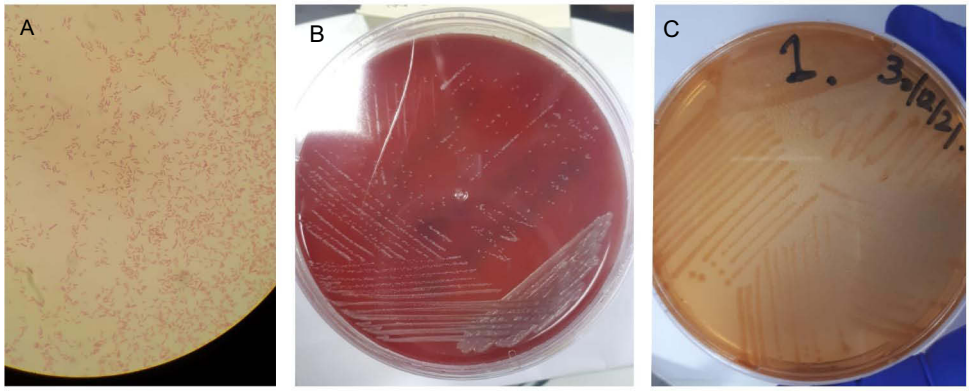

Figure 1 (**A**) *Ralstonia mannitolilytica* in Gram stain; (**B**) *Ralstonia pickettii* 72 hours culture on blood agar; (**C**) *Ralstonia mannitolilytica* 72 hour culture on MacConkey plate.

platforms may give unidentifiable results for *Ralstonia* in the case of absence of reference spectra within the used database.[13] The *Ralstonia pickettii* lineage comprises of *Ralstonia insidiosa, Ralstonia mannitolilytica, Ralstonia pickettii, Ralstonia solanacearum*, and *Ralstonia syzygii*, of which *Ralstonia pickettii* was described as the most frequently reported species in human infections. An older report in Latin America has attributed all cases of 16 BSI to *R. pickettii* where no *R. mannitolilytica* was isolated from blood.[14] The experience of our institution during the COVID-19 pandemic illustrated predominance of *R. mannitolilytica* based on MALDI-TOF identification.

The accurate speciation of *Ralstonia* genus is crucial for the correct clinical management, as the species show different antimicrobial susceptibility patterns (Table 3). Sader et al found *R. pickettii* strains more susceptible to piperacillin-tazobactam than *R. mannitolilytica* which is consistent with our data.[15] Susceptibility of *Ralstonia* isolates in this case series to fluoroquinolones and trimethoprim-sulfamethoxazole is supported by other studies.[9] *Ralstonia pickettii* produces the chromosomally encoded class D beta-lactamase OXA-22, which confers reduced susceptibility to beta-lactam antibiotics such as cephalosporins. Additionally, it is capable of producing the chromosomal beta-lactamase OXA-60 that hydrolyzes imipenem.[16,17] OXA-22 and OXA-60 beta lactamases have been recently reported in environmental strains of *Ralstonia*.[17] To our knowledge, this is the first

Table 3 Susceptibility Patterns for 4 *Ralstonia* Spp. Isolated from Bloodstream Infections

Antibiotic	Susceptible no (%)
Gentamicin	1 (25%)
Amikacin	1 (25%)
Ciprofloxacin	3 (75%)
Levofloxacin	3 (75%)
Piperacillin-tazobactam	1 (25%)
Ceftazidime	1 (25%)
Cefepime	2 (25%)
Ceftazidime-avibactam	1 (25%)
Imipenem	2 (50%)
Meropenem	2 (50%)
Trimethoprim- sulfamethoxazole	3 (75%)

report of OXA-48 among human *Ralstonia* spp (Table 2). This can impose a pending threat of hospital associated infections by the organism that are hard to treat since *Ralstonia* spp. have been implicated in nosocomial outbreaks.[18,19]

Conclusion

In conclusion, this case series highlights the challenges in the treatment of BSI caused by *Ralstonia*, and the difficulties that might be encountered by clinical microbiology laboratories in reporting this infrequently isolated species. Although their sources were not tracked, the occurrence of multiple cases over 2 years dictates stringent infection control measures. The tested β-lactams demonstrated an overall poor potency against the organism while fluoroquinolones and sulfonamides appear to be active in vitro against the pathogen. To our knowledge, this is the first reported series of BSI relating to *Ralstonia* during the COVID-19 pandemic in which OXA-48 is detected. More clinical data are required to further determine the emerging role of *Ralstonia* in invasive infections and the management of BSI caused by the organism.

Ethical Approval

Informed consent to publish was obtained from all cases to publish their cases. No additional approval was needed for these observational, non-interventional case reports. The institution operates in accordance with the 1964 Helsinki declaration and its later amendments.

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Disclosure

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