

A case of persistent bacteraemia by *Ralstonia mannitolilytica* and *Ralstonia pickettii* in an intensive care unit

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Abstract: The *Ralstonia spp.* genus is a group of non-fermentative, Gram-negative bacteria often resistant to many antibiotics, which are emerging as opportunistic pathogens frequently associated with infections in hospital settings. We present herein a case of combined *R. pickettii* and *R. mannitolilytica* persisting and relapsing bacteraemia, possibly caused by a septic arterial thrombosis secondary to the rupture of an internal carotid artery aneurysm. Microbiology studies showed that both *Ralstonia* isolates produced biofilm and carried class D oxacillinase genes. When confronted with infections caused by members of the *Ralstonia* genus, identification to the species level is crucial for correct clinical management, as the two species show different antibiotic susceptibility patterns.

Keywords: *Ralstonia pickettii*, *Ralstonia mannitolilytica*, bacteraemia, endovascular infection

Background

Members of the *Ralstonia* genus are emerging opportunistic pathogens that include *R. pickettii*, *R. mannitolilytica*, *R. solanacearum*, and *R. insidiosa*. *R. pickettii* and *R. mannitolilytica* have been involved in a wide spectrum of infections in hospital settings.¹⁻⁴ Frequent reports of infections associated with these non-fermenting Gram-negative rods, as well as their resistance to many classes of antibiotics, have determined a rise in attention for these pathogens in recent years.⁵⁻⁷

We describe herein a very unusual case of persisting and relapsing bacteraemia caused by both *R. pickettii* and *R. mannitolilytica*, probably secondary to an endovascular infection.

Case report

A 46-year-old female with a medical history of breast implants and thyroidectomy was admitted on 25 March 2018 to the Emergency Department of the Policlinico Umberto I in Rome, Italy, for headache, vomiting and neurological deterioration with hyposthenia of the left hemysome. On admission, a cerebral CT scan showed an intraparenchymal hemorrhage of the right frontoparietal region. Magnetic resonance imaging (MRI) of the brain (with gadolinium) evidenced the rupture of an internal carotid artery aneurysm. After emergency evacuation of the haematoma and clipping of the aneurysm, the patient was transferred to the intensive care unit (ICU). On day 6, the patient developed fever up to 38.5 °C, and was treated for

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5 days with intravenous gentamicin for an *Escherichia coli* urinary tract infection. On day 11, fever up to 39 °C reappeared without hemodynamic deterioration and no worsening of respiratory exchanges. Blood examinations showed neutrophilic leukocytosis, thrombocytopenia, and a procalcitonin value of 1.15 ng/mL (normal value <0.5 ng/mL). Multiple blood cultures revealed the growth of *R. mannitolilytica* and *R. pickettii*. Laboratory results showed that both isolates were susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole, however fever persisted despite targeted therapy with intravenous ciprofloxacin (400 mg tid). On day 19, additional blood cultures were performed, which became positive for *Candida parapsilosis*, *R. pickettii* and *R. mannitolilytica*. Cultures of the removed central venous catheter, respiratory secretions, urine and rectal swab were all negative for *Ralstonia* spp. A transthoracic echocardiogram showed no signs of cardiac vegetations and funduscopic examinations were also negative. Conversely, MRI showed thrombosis of the internal carotid artery upstream of the surgical clip, which required anticoagulant therapy with enoxaparin 6000 IU bid for three months. Clearance of candidemia was obtained after 4 days of antifungal therapy; trimethoprim-sulfamethoxazole (5 mg/kg of trimethoprim iv q6 h) was added to the antimicrobial regimen on day 25 because blood cultures were still positive for both *Ralstonia* species. The antibiotic treatment was discontinued on day 40. However, *R. pickettii* and *R. mannitolilytica* bacteraemia relapsed a week later and the patient was again treated with a six-week course of trimethoprim-sulfamethoxazole and ciprofloxacin. On day 52, the patient underwent cranioplasty surgery without complications. On day 80, the patient developed fever, and *R. pickettii* was once again grown from blood cultures. Ciprofloxacin (400 mg iv tid) was administered; a progressive clinical improvement was observed and the patient was transferred to a rehabilitation centre where she completed an eight-week therapy course. No further relapse of *Ralstonia* infection was observed after a six-month follow up.

Culture and characterization of strains

Blood cultures were incubated in a BACT/ALERT[®] VIRTUO[®] system (bioMérieux, Marcy-l'Étoile, France) at the Clinical Microbiology laboratory of the Policlinico Umberto I in Rome, Italy. Positive blood cultures were seeded on solid media for 48 hrs, and colony identification

was performed using the Vitek-2 automated system (bioMérieux), which identified the *Ralstonia* genus. The MALDI TOF MS (bioMérieux) technique on the other hand yielded a rapid and accurate species-level identification of two distinct species: *R. mannitolilytica* and *R. pickettii* (confidence values of 99%). These identification results were subsequently confirmed by 16S rDNA gene sequencing.

Antimicrobial susceptibility was performed by the broth microdilution method (MicroScan WalkAway plus, Beckman Coulter, Milan, Italy). As there are no EUCAST susceptibility breakpoints available for *Ralstonia* spp., MIC results were interpreted using the criteria used for *Pseudomonas* spp and *Acinetobacter* spp. for trimethoprim-sulfamethoxazole.⁸ As shown in Table 1, the two *Ralstonia* species showed different resistance profiles: *R. mannitolilytica* was resistant to β -lactams and aminoglycosides, but susceptible to piperacillin/tazobactam, while *R. pickettii* was resistant to piperacillin/tazobactam and most of the β -lactams, but susceptible to imipenem and aminoglycosides. Both strains were negative for the presence of carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}, and *bla*_{VIM}), as determined by RT-PCR (GeneXpert, Cepheid, USA).

Antimicrobial resistance genes were also investigated by Whole Genome Sequencing (WGS) using the Ion Torrent GSS5 platform (Life Technologies, Carlsbad, California, USA) by constructing single-end libraries

Table 1 MIC values for the *Ralstonia* spp. clinical isolates described in this study

Antibiotic	Antibiotic MIC values (mg/L) ^a	
	<i>R. pickettii</i>	<i>R. mannitolilytica</i>
Amikacin	4 (S)	32 (R)
Aztreonam	>16 (R)	>16 (R)
Ceftazidime	24 (R)	12 (R)
Cefepime	32 (R)	12 (R)
Ciprofloxacin	0.047 (S)	0.125 (S)
Ertapenem	>32 (R)	>32 (R)
Gentamicin	2 (S)	16 (R)
Imipenem	3 (S)	12 (R)
Levofloxacin	0.125 (S)	0.38 (S)
Meropenem	>32 (R)	>32 (R)
Netilmicin	2 (S)	>8 (R)
Piperacillin	>64 (R)	16 (S)
Piperacillin/tazobactam	32/4 (R)	12/4 (S)
Trimethoprim/sulfamethoxazole	≤1/19	≤1/19 (S)
Tobramycin	<2 (S)	>8 (R)

Notes: ^aMIC were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for *P. aeruginosa*.

Abbreviations: R: Resistant; S: Susceptible; MIC: Minimal inhibitory concentration.

with an average length of 200bp, according to manufacturer's instructions. All raw reads generated were submitted to the Sequence Read Archive (SRA) under the BioProject accession number SRP154097.

The resistance genes were extracted from the WGS data using the ResFinder v3.0 webserver (<http://www.genomicepidemiology.org>). The minimum percentage of sequence identity was set at 100%, with an alignment length of >98%. Both strains carried the resident narrow-spectrum oxacillinase *bla*_{OXA-22}; *R. pickettii* also harboured *bla*_{OXA-60}, an inducible oxacillinase with carbapenem-hydrolyzing property. In addition to the *bla*_{OXA-22} gene, *R. mannitolilytica* showed the presence of the intrinsically species-specific class D oxacillinase *bla*_{OXA-443} and *bla*_{OXA-444} genes, and the serin-hydrolase class C family beta-lactamase (GenBank Accession number WP_045219476). Biofilm production capacity was evaluated by BioTimer-resazurin assay (Canvax Biotech, Spain) as previously described.⁹ Both isolates produced biofilm in a dose-dependent manner with bacterial concentrations as low as 75 live cells/mL.

Discussion

Emerging opportunistic pathogens such *R. pickettii* and *R. mannitolilytica* have been shown to cause several infections, especially in immunosuppressed patients. *R. pickettii* was recognized as a causative agent of infection of the seminal tract, of bones and joints (including prosthetic joints), as well as a cause of infective endocarditis, severe pneumonia and fulminant sepsis.^{10–14} *R. mannitolilytica* on the other hand has been described as a cause of recurrent ventricular-atrial shunt-associated meningitis.⁴ The ability of these bacteria to cause localized and systemic infections is likely due to their ability to produce biofilm, which in turn is key to their survival in the environment, their evasion of the host's immune response and their frequent antibiotic resistance.^{15,16} The clinical presentation of both species is also similar, and both have been described as agents of bacteraemia, especially in high-risk patients.^{7,17,18}

To the best of our knowledge, this is the first report of persisting and relapsing bacteraemia caused by two distinct *Ralstonia* species in an immunocompetent patient. Our case concerns a patient without any apparent comorbidity or predisposing condition who developed the first episode of bacteraemia by the two *Ralstonia* species six days after the clipping of an internal carotid aneurysm. One possibility is that the infection was acquired following contamination during or after the neurosurgical clipping procedure.

Indeed, *Ralstonia* bacteria have been associated with infections in hospital settings due to their ability to survive in liquid media (including saline and chlorhexidine with 0.05% aqueous solutions) and on hospital devices.¹⁹ In our case, environmental samples of air or from inanimate surfaces taken monthly in the operating rooms and in all areas where the patient had been treated were negative for *Ralstonia* spp; this is not surprising as even in cases of well documented hospital outbreaks the environmental origin was not identified.^{20,21} The case we have described was also the only occurrence in the hospital, supporting the hypothesis that no environmental contamination had taken place. Furthermore, an infection related to the use of an intravascular device was also ruled out, since cultures of the removed central venous catheter did not show any growth of *Ralstonia* species. An alternative hypothesis was based on the study by Ziganshina et al,²² who demonstrated that members of the genera *Ralstonia* are one of the most significant taxa identified in atherosclerotic plaques removed during surgery. Thus, we could speculate that either the aneurysm rupture or the subsequent neurosurgical management might have provided access to an anatomically protected site. Unfortunately, atheroma material was not available to confirm this hypothesis. Regardless of the source of the infection, a combination of arterial thrombosis and biofilm-related chronic contamination, could have represented a key factor for the persistence and relapse of the *Ralstonia* spp. bacteraemia, which occurred despite an appropriate and long-term antibiotic therapy.^{23,24}

The antimicrobial treatment and management of *Ralstonia* spp. infections is challenging, firstly because of the difficulty in correctly identifying and differentiating between *Ralstonia* spp. members using routine biochemical methods.^{7,17} Although the 16S rDNA is described as the reference method, in our case the mass spectrometry yielded a rapid and accurate species-level identification, in accordance with other studies.^{21,25} *Ralstonia* species are also resistant to different antimicrobial agents, mainly β -lactams (including carbapenems) given the presence of the class D β -lactamases *bla*_{OXA-22} and *bla*_{OXA-60} genes. As our WGS results show, the close aminoacid identities of OXA-444 protein to the OXA-60 and the presence of the serin-hydrolase class C beta-lactamase, may explain the carbapenem resistance (imipenem included) of the *R. mannitolilytica* isolate. These enzymes, in association with different mechanisms such as porin deficiency or over-expression of efflux pumps, may confer resistance to multiple classes of antibiotics.^{10,17}

Aggregate data obtained from a literature review revealed a notable heterogeneity in the percentage of antibiotic resistance, as well as differences in the antibiotic susceptibility profiles of the two species. Although our analysis included only two isolates (very few compared to the literature data of 35 strains shown in Table 2) we did observe antibiotic resistance discrepancies regarding third generation cephalosporins and imipenem. Our patient was treated with ciprofloxacin and trimethoprim-sulfamethoxazole, both considered first-choice antibiotics. This turned out to be the best management of the patient; other treatment recommendations include third-generation cephalosporins or carbapenems, which would probably have not been able to eradicate the infection.¹¹ Despite *Ralstonia* spp. are not recognized as major pathogens, their multidrug resistance, biofilm formation potential and ability to survive in the environment are factors that should never be underestimated. Clinicians and microbiologists should pay attention to the potential of these opportunistic bacteria able to cause severe bloodstream infections.

Table 2 Antimicrobial resistances identified in *R. pickettii* and *R. mannitolilytica* clinical isolates, obtained from a literature review

Antimicrobial	<i>R. pickettii</i> ^a	<i>R. mannitolilytica</i> ^b
Amikacin	9/16 (56.3%)	11/12 (91.7%)
Ampicillin	1/3 (33.3%)	7/8 (87.5%)
Ampicillin-sulbactam	Not tested	2/6 (33.3%)
Aztreonam	7/9 (77.8%)	9/11 (81.8%)
Cefepime	2/9 (22.2%)	5/10 (50%)
Ceftazidime	6/14 (42.9%)	9/14 (64.3%)
Colistin	4/4 (100%)	3/3 (100%)
First generation cephalosporins	2/4 (50%)	4/4 (100%)
Fluoroquinolones	4/17 (23.5%)	6/15 (40%)
Gentamicin	12/14 (85.7%)	14/15 (93.3%)
Imipenem/cilastatin	2/13 (15.4%)	7/11 (63.6%)
Meropenem	5/9 (55.6%)	9/9 (100%)
Piperacillin/tazobactam	1/14 (7.1%)	3/14 (21.4%) ^c
Tetracyclines	0/4 (0%)	1/0 (100%)
Trimethoprim/sulfamethoxazole	1/12 (8.3%)	1/12 (8.3%)

Notes: 19 strains of *R. pickettii* and 16 of *R. mannitolilytica* were analysed; each agent or agent class sensitivity was evaluated in 65% for *R. mannitolilytica* (median, IQR 40.6–81.2%) and in 66.7% (median, IQR 48.3–83.3%) isolates of *R. pickettii*. The data report the number of resistant strains on the absolute number and the relative percentage in brackets. Isolates with intermediate susceptibility were considered as susceptible. ^aReferences for *R. pickettii*.^{1,2,6,10–14,18,20,31–38} ^bReferences for *R. mannitolilytica*.^{3–5,17,21,26–30} ^cOne strain was resistant to piperacillin and susceptible to piperacillin/tazobactam.

Ethical statement

The patient provided written informed consent to describe the case details, and no institutional approval was needed for publication.

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Author contributions

All authors contributed towards data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in regard to this work.

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