Ralstonia mannitolilytica bacteraemia: a case report and literature review

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SUMMARY

Ralstonia mannitolilytica is a difficult-to-diagnose, aerobic, Gram-negative bacillus, mainly causing healthcare infections in immunocompromised hosts. We report the first case of *R. mannitolilytica* bacteraemia in a kidney transplant recipient. Identification of *R. mannitolilytica* was finally performed by 16S rRNA gene sequencing. All cases of *R. mannitolilytica* bacteraemia reported in the English language literature over the past 20 years are reviewed to alert clinicians to the epidemiological, clinical, diagnostic, prognostic and microbiologic features of this emerging pathogen.

Keywords: *Ralstonia mannitolilytica*, catheter-related bacteraemia, catheter-associated bacteraemia, gram-negative rods; kidney transplant recipient.

INTRODUCTION

The genus *Ralstonia* refers to a Gram-negative, oxidase-positive, non-fermentative rods typically distributed in environmental habitats such as water, soil and plants [1]. It includes 13 species and *Ralstonia pickettii* has been implicated in the majority of the infections. *Ralstonia mannitolilytica*, previously known as *Pseudomonas thomasii* or *R. pickettii* biovar 3/ˆthomasii´, has been deemed to be involved in nosocomial outbreaks secondary to medical devices, equipment, water or parenteral solutions contamination [2, 3]. It is known as an opportunist human pathogen, infecting mainly immunocompromised hosts [1]. It is also supposed to be a predictor of poor outcomes in cystic fibrosis patients and it has been isolated in newborns and in patients with solid cancer, haematological disease, ventriculoatrial draining for hydrocephalus, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and scleroderma [4]. Meningitis, peritonitis, osteomyelitis, hemoperitoneum infection, urinary tract infection and drain site infection in kidney transplant recipient cases have been reported [1-10]. Similarly to *R. pickettii*, and due to its ability to create biofilm, *R. mannitolilytica* has been postulated to be also involved in bacteraemia but limited data are reported in literature [1-3, 4-10]. This is probably due to the problematic identification of *R. mannitolilytica* that often is confused with *R. pickettii*, *Burkholderia cepacia* complex and *Pseudomonas fluorescens* [1, 9]. A case of *R. mannitolilytica* central venous catheter related bacteraemia in a kidney transplant recipient together with a comprehensive literature review of *R. mannitolilytica* bacteraemia is presented to help clinicians to be aware about epidemiological, clinical, diagnostic, prognostic and microbiologic features of this emerging pathogen.

CASE REPORT

In November 2017, a 44-year-old man with end-stage kidney disease on haemodialysis underwent kidney transplant in our hospital. The patient had a medical history of mesangial proliferative glomerulonephritis and hypertension.

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Transplant surgery had no complications and the patient started *ab-initio* immunosuppressive regimen with tacrolimus, mycophenolate mofetil and prednisone.

On hospital day 11, the patient presented with fever (T 39ºC) and shivering. The physical examination was unremarkable. Blood analysis showed white blood cell count 17,000 per mm^3

Table 1 - Literature review of *R. mannitolilytica* susceptibility in bacteraemia cases over the past 20 years (Block et al. 2013 and Coman et al. 2016 were not listed due to not reporting antimicrobial susceptibility).

	Boattini et al. 2018	*Vaneechoutte et al. 2001*	*Daxboeck et al. 2005*	*Grobner et al. 2007*	*Soloaga et al. 2011*	*Liu et al. 2016*			*Lim et al. 2017*	*Lucarelli et al. 2017*
According to	EUCAST[1]	n.a.	CLSI	CLSI	n.a.	CLSI			n.a.	EUCAST[3]
Minimal inhibitory concentration µg/mL (Antimicrobial susceptibility)										
AM	n.a.	(R)	n.a.	(R)	n.a.	>32 (R)	>32 (R)	> 64 (R)	n.a.	n.a.
SAM	n.a.	n.a.	n.a.	(S)	n.a.	≤2 (S)	16 (I)	64 (R)	n.a.	n.a.
PIP	>16 (R)	(S)	n.a.	(S)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TZP	16 (R)	n.a.	n.a.	(S)	16 (S)	≤4 (S)	≤ 4 (S)	≤ 4 (S)	(S)	(S)
C_T	6 (R)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CZ	n.a.	n.a.	n.a.	n.a.	n.a.	32 (R)	>64 (R)	> 64 (R)	n.a.	n.a.
CAZ	4 (S)	(S)	n.a.	n.a.	n.a.	2 (S)	> 64 (R)	> 64 (R)	(S)	(R)
CXM	n.a.	(S)	n.a.	(S)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CTX	1 (S)2	(S)	n.a.	(S)	n.a.	4 (S)	2 (S)	4 (S)	n.a.	n.a.
FEP	1 (S)	n.a.	(S)	n.a.	n.a.	>1 (R)	32 (R)	8 (R)	n.a.	n.a.
IPM	2 (S)	(S)	n.a.	n.a.	8 (R)	>1 (R)	>16 (R)	> 16 (R)	n.a.	n.a.
MEM	>32 (R)	n.a.	n.a.	> 256 (R)	> 32 (R)	n.a.	n.a.	n.a.	(R)	(R)
CIP	0.094 (S)	(S)	(S)	(S)	0.125 (S)	0.5 (S)	> 4 (R)	> 32 (R)	n.a.	(R)
LVX	0.047 (S)	(S)	n.a.	(S)	n.a.	0.5 (S)	> 8 (R)	> 64 (R)	n.a.	n.a.
AN	2 (S)	n.a.	(R)	n.a.	n.a.	4 (S)	>64 (R)	> 128 (R)	(R)	(R)
GM	0.19 (S)	(R)	(R)	(R)	n.a.	2 (S)	>16 (R)	> 256 (R)	(R)	(R)
TM	n.a.	n.a.	n.a.	(R)	n.a.	2 (S)	>16 (R)	> 64 (R)	n.a.	n.a.
SXT	0.032 (S)2	(S)	n.a.	(S)	0.1 (S)	≤20 (S)	≤20 (S)	≤ 20 (S)	n.a.	n.a.
ATM	n.a.	(R)	n.a.	n.a.	n.a.	8 (S)	>64 (R)	> 256 (R)	n.a.	n.a.
TGC	1.5 (S)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

AM: Ampicillin; SAM: Ampicillin-Sulbactam; PIP: Piperacillin; TZP: Piperacillin+Tazobactam; C_T: Ceftolozane/Tazobactam; CZ: Cefazolin; CAZ: Ceftazidime; CXM: Cefuroxime; CTX: Cefotaxime; FEP: Cefepime; IPM: Imipenem; MEM: Meropenem; CIP: Ciprofloxacin; LVX: Levofloxacin; AN: Amikacin; GM: Gentamicin; TM: Tobramycin; SXT: Sulfamethoxazole- Trimethoprim; ATM: Aztreonam; CS: Colistin; TGC: Tigecycline;
I: Intermediate; S: Susceptible; R: Resistant; n.a.: not available;
[1]According to Pseudomonas spp. European Committee on Antimicrobial Susceptibility Testing Clinical Breakpoint (2018) and
[2]According to Enterobacteriacae European Committee on Antimicrobial Susceptibility Testing Clinical Breakpoint (2018) for SXT and CTX;
[3]According to Pseudomonas spp. European Committee on Antimicrobial Susceptibility Testing Clinical Breakpoint (2018).

[4,500-11,000], neutrophilia of 91% and C-reactive protein 175 mg/L [<0.5]. Chest-X ray showed no lung infiltration. Abdominal ultrasound revealed no alterations suggestive for infectious foci. Urine culture was negative. Transoesophageal echocardiography ruled out endocarditis. Two pairs of aerobic and anaerobic blood cultures were drawn simultaneously, one through the CVC and the other peripherally. Cefepime (2 g every 24 h, i.v.) and daptomycin (500 mg every 24 h, i.v.) were started. After 17 hours, Gram staining showed gram negative rods in the aerobic blood culture drawn through the CVC. Daptomycin was stopped and the CVC was removed. After 31 hours Gram staining showed Gram negative rods in the peripherally drawn aerobic blood culture too. After 41 hours from the initiation of the blood cultures, domed, smooth and oxidase positive colonies grew on blood agar plates and non-lactose-fermenter colonies grew on MacConkey agar plates.

Biochemical identification showed acidification of D-arabitol and mannitol and lack of nitrate reduction. Microscan Walkaway 96 Plus (Beckman Coulter, Nyon, Switzerland) and Phoenix (Becton Dickinson, Milan, Italy) automated systems were not able to identify the isolated agent.

Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (VITEK MS system - BioMérieux, Marcy L'Étoile, France) provided identification of *Ralstonia pickettii* (confidence values of 99.9%).

The isolate subsequently underwent 16S rRNA gene sequencing that identified *R. mannitolilytica* (100% match with *R. mannitolilytica* strain M24, GenBank accession LN890110).

The *in vitro* susceptibility of the isolate was assessed with Etest. Results are reported in Table 1.

Following the results of susceptibility testing, cefepime was continued for 14 days. Patient was discharged on day 21 in good clinical condition and he is still being followed in Nephrology out-patient clinic of our hospital.

In order to elucidate a source of *R. mannitolilytica* infection and to avoid outbreaks, a comprehensive environmental survey including cultures of in-use parental solutions, filled syringes, disinfectants, medical devices and water was performed in the wards to which the patient had been admitted but yielded negative results. All microbiologic data of our hospital were reviewed, but no *Ralstonia spp* have been matched in the last three years.

DISCUSSION

Bacteraemia, central venous catheter associated bacteraemia (CVCaB) and central venous catheter related bacteraemia (CVCrB) are deemed to be important causes of morbidity, mortality and high additional costs, especially in hospital-acquired infection. Bacteraemia defines the presence of a potential pathogenic agent, cultured from at least one blood culture, in a patient with sepsis. CVCaB refers to a bloodstream infection in a patient with an intravascular device and in the absence of a site-specific infection. CVCrB delineates the presence of >1 positive blood culture drawn peripherally in a patient with sepsis, central venous catheter (CVC) and no apparent other source for the bloodstream infection, except the CVC. Moreover, in CVCrB one of the following should be also present: a positive semi-quantitative (>15 colony forming units/catheter segment) or quantitative (>10^2 colony forming units/catheter segment) CVC tip culture for the same pathogen isolated from the peripheral blood culture; a >3:1 colony forming units/mL CVC versus peripheral of simultaneous quantitative blood cultures *ratio*; a >2 hours time to positivity of a non-quantitative blood culture drawn simultaneously from the CVC and peripherally.

Bacteraemia, CVCaB and CVCrB are associated with a high mortality and morbidity outcomes but data about systematic approach to the diagnosis, treatment and management of *R. mannitolilytica* bloodstream infections are lacking.

A comprehensive literature review of *R. mannitolilytica* bacteraemia was performed and 9 papers, reporting the total of 35 cases, have been found [2-10]. Epidemiological, clinical, diagnostic, prognostic features of all cases of *R. mannitolilytica* bacteraemia over the past 20 years are provided in Table 2.

We highlight that *R. mannitolilytica* bacteraemia has been exclusively identified in immunodeficient patients in nosocomial setting with no apparent age or gender predilection. Only 3 *R. mannitolilytica* bacteraemia outbreaks have been reported [5, 6, 8].

Surgical infectious complications, parenteral drug treatment, haemodialysis session and septic shock in cystic fibrosis patients have been included as reasons for hospital admission.

The incidence of *R. mannitolilytica* infections may be underreported due to misidentification on actual automated laboratory platforms.

Table 2 - Epidemiological and clinical features of *R. mannitolilytica* bacteraemia over the past 20 years.

Reference	*Sex/Age (years)*	*Setting*	*Co-morbidity*	*Reason for admission*	*Identification system*	*Source of infection*	*Outcome*
Vaneechoutte et al. 2001	F/38	Nosocomial	Cavernous hemangioma	Meningitis following neurosurgery	API 20NE (bioMérieux, Marcy L'Ètoile, France)	CVCrB	Recovery
Daxboeck et al. 2005	25 cases	Nosocomial outbreak	Not reported	Not reported	16S rRNA sequencing	Not reported	Recovery
Grobner et al. 2007	F/41	Nosocomial outbreak	Acute myeloid leukemia	Not reported	API 20NE and Vitek II (bioMérieux, Nurtingen, Germany), 16S rDNA sequencing	CVCaB	Recovery
	M/21		Acute lymphoblastic leukemia	Not reported		CVCaB	
	M/45		Acute lymphoblastic leukemia	Not reported		CVCrB	
	M/7		Scleroderma	Stem cell transplantation		CVCaB	
	F/67		B-cell Non-Hodgkin Lymphoma	Not reported		CVCaB	
Soloaga et al. 2011	M/26	Nosocomial	Diabetes, hipothyroidism, Histiocytosis X	Histiocytosis X treatment	API 20NE, Vitek Compact 2, Vitek 1 (bioMérieux, Marcy L'Ètoile, France)	CVCrB	Recovery
Block et al. 2013	New-born	Nosocomial	Premature infant	Preterm birth	API 20NE and VITEK MS (bioMérieux, Marcy L'Ètoile, France), 16S rRNA sequencing	Not reported	Recovery
Liu et al. 2016	F/74	Nosocomial	Diabetes, Hypertesion, gastric T-cell lymphoma	Cancer surgery	Vitek Compact 2 (bioMérieux, Marcy L'Ètoile, France)	CVCaB	Recovery
	M/56		Gastric Carcinoma	Cancer surgery		CVCaB	
	F/55		Diabetes, Hypertesion, hepatic hemangioma	Hepatic lobectomy		CVCaB	
Coman et al. 2016	F/39	Nosocomial	Cystic fibrosis	Septic shock, lung abscess	Not reported	Pneumonia	Death
	F/19			Septic shock		Pneumonia	Death
Lim et al. 2017	F/65	Nosocomial	Diabetes, Hypertesion, end stage renal disease on dialysis, ischaemic heart disease	Dialysis session	Not reported	CVCaB	Recovery
Lucarelli et al. 2017	22 cases	Nosocomial outbreak	Solid cancer	Cancer treatment	Vitek 2 (bioMérieux, Florence, Italy), 16S rDNA sequencing	CVCrB (12 patients) CVCaB (6 patients) Undetermined (4 patients)	Recovery
Boattini et al. 2018	44/M	Nosocomial	End stage renal disease	Kidney transplant	16S rRNA sequencing	CVCrB	Recovery

CVCrB: Central venous catheter-related bacteraemia; CVCaB: Central venous catheter-associated bacteraemia.

Recent introduction of MALDI-TOF mass spectrometry could improve the possibilities to phenotypically identify this unusual pathogen but biochemical identification, growth on *Burkholderia cepacia* selective medium and 16S rRNA gene sequencing continue to be the only reliable identification systems [9]. Therefore *R. mannitolilytica* should be considered a difficult-to-diagnose pathogen.

R. mannitolilytica in bacteraemia cases has been detected by several automated identification systems and gene sequencing has been performed in the 50% of cases. An 83.3% rate of CVCaB (30 of 36 *R. mannitolilytica* bacteraemia cases) and a 44.4% rate of CVCrB (16 of 36 *R. mannitolilytica* bacteraemia cases) have been described. Few data about semi-quantitative or quantitative culture tip, colony forming units/mL CVC vs peripheral blood cultures *ratio* and time to positivity have been reported; therefore CVCrB are probably underreported.

Despite of a high morbidity rate, *R. mannitolilytica* bacteraemia showed good prognosis, except in cystic fibrosis patients.

Limited data about antimicrobial susceptibility are reported too. *R. mannitolilytica* is known to be intrinsically resistant to colistin and *R. mannitolilytica* susceptibility in bacteraemia cases over the past 20 years is provided in Table 1.

Variable *R. mannitolilytica* susceptibility to ceftazidime, cefepime and carbapenem and aminoglycosides resistance have been reported. *R. mannitolilytica* seems to easily gain antimicrobial resistance presenting a susceptibility profile not far to a multidrug resistant agent: it has been postulated that broad spectrum antimicrobial treatment should contribute to increase *R. mannitolilytica* strains resistance [2]. However, where reported, all *R. mannitolilytica* involved in bacteraemia cases showed susceptibility to sulfamethoxazole-trimethoprim and most of the strains appeared susceptible to fluoroquinolones, cefotaxime and piperacillin-tazobactam. Limited data about minimal inhibitory concentration (MIC) values and Antimicrobial Susceptibility Testing Clinical Breakpoint reference systems have been reported. We reported antimicrobial susceptibility testing according to *Pseudomonas spp.* EUCAST 2018 and *Enterobacteriacae* EUCAST 2018 only for sulfamethoxazole-trimethoprim and cefotaxime [6].

We highlight the considerable need to report *R. mannitolilytica* MIC to contribute to the future creation of specific clinical breakpoints. Equally, we report the considerable need to test *R. mannitolilytica* susceptibility for sulfamethoxazole-trimethoprim because of it has been successfully used as treatment in literature.

Further studies are needed to have a greater understanding of *R. mannitolilytica* epidemiology in bacteraemia cases to help clinicians to rapidly address antimicrobial therapy, to understand its prognostic value and to dispose environmental survey for infection control.

Conflicts of interest

None.

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