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A pan-resistant *Myroides odoratimimus* catheter-related bacteremia in a COVID-19 patient and review of the literature

Acta Microbiologica et Immunologica Hungarica

69 (2022) 2, 164–170

DOI:
10.1556/030.2022.01702

RESEARCH ARTICLE

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Received: January 3, 2022 • Accepted: March 4, 2022
Published online: March 21, 2022

ABSTRACT

Myroides spp. are opportunistic environmental Gram-negative bacteria. These affect mostly immunocompromised hosts and generally lead to soft tissue, and urinary tract infections. Bacteremia most commonly develop secondary to soft tissue or catheter related infections and may lead rarely to mortality. *Myroides* spp. are generally suscetible to fluoroquinolones, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, carbapenems or tetracyclines however, pan-resistant isolates and multiple resistance genes have been reported in clinical isolates of *Myroides* spp. We report a pan-resistant *Myroides odoratimimus* bacteremia in a patient with severe COVID-19 ending with fatality and in this context a review of reported *Myroides* bacteremias are also described.

In this study, a 64-year old male patient with history of coronary artery bypass was admitted to ICU with severe COVID-19 pneumonia accompanied by pneumomediastinum and pneumopericardium. Continous renal replacement therapy and extracorporeal membraneous-oxygenation were initiated due to acute renal failure and persistent hypercarbia/hypoxia, respectively. Within four weeks of hospitalization various episodes of bacteremia developed and multiple antibiotics were used. On the 5th week of follow-up, acute phase reactants increased and empirical broad spectrum antibiotics were initiated. Blood culture revealed Gram-negative rods. The patient became hypotensive and despite maximum medical care he was lost due to cardiac arrest. *M. odoratimimus* was identified by MALDI-TOF and the bacterium was pan-resistant. According to Center for Genomic Epidemiology results the strain was identified as *M. odoratimimus* PR63039 and the genome analysis revealed antibiotic resistance genes associated with resistance to beta-lactams ($bla_{\text{OXA-347}}$, $bla_{\text{MUS-1}}$, $bla_{\text{EBR-1}}$), tetracyclines (*tetX*), sulfonamides (*sul2*), macrolides (*ereD*), (*ermF*).

KEYWORDS

Myroides odoratimimus, pan-resistant, catheter-related bacteremia

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INTRODUCTION

Myroides spp., previously described in the genus *Flavobacterium*, were reclassified in 1996 and two species *Myroides odoratus* and *Myroides odoratimimus* were identified [1, 2]. Later, nine species have been isolated: *Myroides pelagicus* sp. nov, *Myroides profundi* sp. nov, *Myroides marinus* sp. nov, *Myroides phaeus* sp. nov, *Myroides injenensis*, *M. guanonis* sp.

nov, *Myroides xuanwuensis* sp. nov, *Myroides indicus* sp. nov, *Myroides* sp. N17-2 [3]. They are found in soil and water. Although they rarely cause infections, skin and soft tissuse infections, bacteremia and urinary tract infections have been reported [1–5]. *Myroides* spp. are aerobic, pale yellow pigmented, non-fermentative, oxidase and catalase positive Gram-negative rods growing on various media including MacConkey agar. They have a characteristic fruity odor and can grow at 18°–37 °C but have no capability to grow at 42 °C. They are immotile, indole and esculin negative, DNase and urease positive and polymyxin resistant. Most of the strains are highly resistant to antibiotics including penicillins, cephalosporins, aminoglycosides, aztreonam and carbapenems [2, 4].

We present a case of pan-resistant catheter related *M. odoratimimus* bacteremia developing in a COVID-19 patient and in this context a review of reported *Myroides* bacteremias.

CASE REPORT

A 64-year old male patient with diagnosis of COVID-19 pneumonia was transferred to the intensive care unit (ICU) of our hospital from an outer center due to respiratory insufficiency at the 12th day of hospitalisation. The patient was conscious, cooperated and was on non-invasive mechanical ventilation on admission. He was administered favipravir (10 days), tocilizumab (400 mg/day, 2 doses) and dexamethasone (8 mg/day, 4 days) for treatment. Glasgow coma score was 15, respiratory rate 36/min and heart rate 66/min. On physical examination diffuse thoracal subcutaneous emphysema was present and crepitations were heard bilaterally on lung auscultation. Oxygen saturation level was 78%, PO2: 35 mmHg and lactic acid: 3.5 mEq/L; the patient was immediately intubated and put in prone position. Thorax CT revealed diffuse alveolar damage secondary to COVID-19 pneumonia, pneumomediastinum and pneumopericardium. Laboratory values on admission were as follows: WBC:17.300/mm^3, lymphocyte: 500/mm^3 platelet: 359.700/mm^3, creatinin: 0.7 mg/dL, total bilirubin: 1.43 mg/dL, AST:37 U/L, ALT:40 U/L, LDH: 824 IU/L (<250), ferritin: 1,391 ng/mL (30–400), D-dimer: 5.42 mg/L (0–0.5), CRP:2,9 mg/L (0–4), procalcitonin:<0.02 ng/mL (0–0.5).

It was learned from his medical history that he had coronary artery bypass surgery 8 years ago. He had been followed-up 39 days in ICU. The patient's general condition deterioriated. On the 14th day of hospitalization continous renal replacement therapy was initiated due to acute renal failure. One week later the patient was supported with extracorporeal membraneous-oxygenation (ECMO) due to persistent hypercarbia and hypoxia. Within 4 weeks of hospitalization four different episodes of bacteremia (*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, carbapenemase producing *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*) and a candidemia developed and these were treated successfully with appropriate therapies. Due to bacteremic episodes the patient was administered piperacillin-tazobactam, clarithromycin, meropenem, vancomycin, cefoperazone-sulbactam, linezolid, colistin, ceftazidime-avibactam, fosfomycin, imipenem, co-trimoxazole empricillay and/or therapeutically. Lastly on the 32nd day of follow-up, the patient's general condition was still bad and the acute phase reactant levels increased again, CRP: from 99 to 248 mg/L (0–5), procalcitonin: 0.072–5.18 ng/mL. Other laboratory values were as follows: WBC: 18.000/mm^3, neutrophile: 16.300/mm^3 lymphocyte: 1,600/mm^3, platelet: 359.700/mm^3, creatinin: 1.09 mg/dL, total bilirubin: 5.14 mg/dL, AST: 104 U/L, ALT:403 U/L, LDH: 914 IU/L, ferritin: >2000 ng/mL, D-dimer: 2.83 mg/L. Meanwhile the patient was under co-trimoxazole therapy for *S. maltophilia* bacteremia since one week. Blood cultures were obtained and colistin and fosfomycin were initiated empirically considering probable nosocomial pathogens. Ten hours later blood culture bottles drawn from central venous line, dialysis' catheter and peripheral vein revealed Gram-negative rods. *M. odoratimimus* was identified by matrix assisted laser desorption and ionization time-of-flight (MALDI-TOF, Bruker Daltonics, Bremen, Germany). The patient became hypotensive and required continuous vasopressor norepinephrine infusion and hydrocortisone treatment. ECMO as well as mechanical ventilation were continued. Despite maximum medical care, the patient was lost due to cardiac arrest.

MICROBIOLOGY

Bacterial identification, antimicrobial susceptibility

Blood cultures (BD BACTEC FX, Becton Dickinson, USA) revealed Gram-negative rods. Pale yellow smooth colonies with fruity odor were isolated from blood, chocolate, and MacConkey agar after 24 h of incubation in aerobic conditions. The isolate was non-lactose fermenting, oxidase and catalase positive, immotile, indole and esculine negative, DNase and urease positive. Antibiotic susceptibility testing was performed with Kirby-Bauer disk diffusion method firstly and confirmed with gradient test method according to EUCAST guidelines and with automatized system (BD Phoenix System, Becton Dickinson, USA) [5]. Polymixin E resistance was also confirmed by disk elution method [6]. The isolate was pan-resistant (Table 1). The mass spectra were analyzed by Bruker Biotyper 3.1 software, IVD Version 8 (DB-7712MSP) library. The isolate was identified as *M. odoratimimus* with reliable score values (2,69).

Resistome analysis

A passage of bacterial strain was taken on Mueller Hinton agar (France) and 24h fresh passage was prepared. From this passage, 2 McFarland suspension was prepared in sterile 0.9% saline, and 200 µl of this suspension was used for nucleic acid isolation. Nucleic acid isolation was performed with the column-based commercial kit (RTP Pathogen Kit, Stratec, Germany) according to the manufacturer's instructions. The amount of DNA obtained was measured by

Table 1. Antimicrobial susceptibility of *M. odoratimimus*

Antimicrobial agents	Disk diffusion (zone diameter)	Gradient test (MIC: µg/mL)	Phoenix (MIC: µg/mL)
Ampicillin	R		>16
Piperacillin-tazobactam	R		>16/4
Cefuroxime	R		>16
Cefotaxime	R		
Cefoxitin	R		
Ceftriaxone			>4
Ceftazidime	R		>8
Cefepime	R		>8
TMP-SXT	R	>32	
Ciprofloxacin	R		>1
Levofloxacin	R	>32	>2
Amikacin	R	>256	>32
Gentamicin	R		>8
Ertapenem	R		>1
Imipenem	R		>8
Meropenem	R		
Tigecycline	R		>2
Doxycycline		8	
Nitrofurantoin	11 mm		
Polymyxin E*			>4/4
Rifampin	13 mm		

MIC: Minimum inhibitory concentration, TMP-SXT: trimethoprim-sulfamethoxazole, R: resistant.
*Resistant by disk elution method [6].

HS dsDNA protocol with a Quibit analyser (Thermo Fisher, USA) and DNA sample was prepared with a final concentration of 2 ng/µL in TE buffer. Bacterial DNA samples were fragmented by taking 50 µl of the prepared sample, 15 s sonication in sonicator and 15 s waiting time (44 cycles). Fragmented DNA was again quantified with Quibit analyser using the HS dsDNA protocol, and the fragmented sample prepared at a final concentration of 1 ng μL^{-1} in TE buffer. This product was used for library construction.

The Nextera XT DNA Library Preparation Kit (Illumina, United States) was used to construct the library. Tagmentation process of DNA fragments obtained with this kit and then barcoding processes were contacted. A library pool was created with an initial concentration of 2 nM and a final concentration of 1.2–1.3 pM, along with other samples to be entered into the device, the prepared pool was loaded into the MiniSeq device (Illumina, USA) and reading processes were performed. Paired end reads data files obtained from the device were uploaded online to KmerResistance 2.2 service provided by Center for Genomic Epidemiology (https://www.genomicepidemiology.org/) and "Species determination on maximum query coverage" was used to search for resistance genes for bacteria. It was analysed to have at least 70% similarity and 10% coverage depth [7–9].

Analysis of *M. odoratimimus* PR63039 genome revealed antibiotic resistance genes associated with resistance to beta-lactams ($bla_{OXA-347}$, bla_{MUS-1}, bla_{EBR-1}), tetracyclines (*tetX*), sulfonamides (*sul2*), macrolides (*ereD*), (*ermF*) (Table 2).

DISCUSSION

Myroides spp. are opportunistic environmental Gram-negative microorganism. They are not included in normal human flora but they may be isolated from urine, soft tissue, sputum, blood and rarely other sites [1–4, 10, 11]. *Myroides* spp. most commonly lead to soft tissue, and urinary infections and *M. odoratus* and *M. odoratimimus* are the most common isolates causing human infections [1, 3, 10]. *Myroides* spp. infections have been reported mainly from China [4]. Although rarely causing infections in human they may also lead to outbreaks [10, 11].

They affect mostly immunocompromised hosts such as those with cirrhosis, diabetes mellitus, malignancies and patients on corticosteroid treatment [1, 12, 13]. *Myroides* spp. generally lead to infections ending with recovery, but rarely may be life-threatining [14, 15].

Based on a review of the cases of bacteremia caused by *Myroides* spp. listed in the U.S. National Institutes of Health's National Library of Medicine (Pubmed), it is noteworthy that bacteremia most commonly develop secondary to soft tissue or catheter related infections [1, 3, 11–28] (Table 3). This case is the 7th catheter related bacteremia reported. The patient was immunocompetent before diagnosis of COVID-19 but during treatment of COVID-19 tocilizumab and corticosteroids were administered. Due to various bacteremic episodes multiple broad spectrum antibiotics were prescribed and all these factors might have led to selection of pan-resistant opportunistic *M. odoratimimus*.

Although isolated *Myroides* spp. are generally susceptible to fluoroquinolones, piperacillin/tazobactam, trimethoprim/sulfamethoxazole carbapenems and tetracyclines [12, 16, 17, 20, 25–29] recently pan-resistant species and presence of metalloenzymes have been reported [30, 31]. Ming et al., in their analysis of pan-resistant *M. odoratimimus* identified resistance genes to tetracyclines (*tetX*), macrolides (*ereB*), lincosamide (*lasE*), sulfonamides (*sul2*, *sul3*), beta-lactams (bla_{MUS-1}, bla_{TUS-1}, bla_{SFB-1}, bla_{SLB-1}, $bla_{OXA-209}$, $bla_{OXA-347}$), chloramphenicol (*cat*) and fluoroquinolones (*parE*) and 18 antibiotic efflux pump-encoding genes [30]. The isolate reported in our study was also pan-resistant and antibiotic

Table 2. Antibiotic resistance genes in the genome of *M. odoratimimus* PR63039

Type	Antibitic resistance gene	Template length
Beta-lactams	$bla_{OXA-347}$_1_ACWG01000053	825
	bla_{MUS-1}_1_AF441286	741
	bla_{EBR-1}_1_AF416700	708
Macrolides	*ereD*_1_KP265721	1,227
Tetracyclines	*tetX*_2_M37699	1,167
Sulfonamides	*sul2*_2_AY034138	816
	*ermF*_3_M17808	801

Table 3. Bacteremias caused by *Myroides* spp. reported in the U.S. National Institutes of Health's National Library of Medicine (Pubmed)

Reference/year	Species	Source	Age/ sex	Host factors	Resistance	Treatment	Outcome
Prieur/1988 [15]	*M. odoratus*	Cellulitis (?)	68/M	Heart failure, diabetes	Susceptible to amoxicillin-clavulanic acid, piperacillin, ticarcillin, and trimethoprim-sulfamethoxazole	Trimethoprim/ sulfamethoxazole	Cured
Hsueh/1988 [16]	*M. odoratus*	Necrotizing fasciitis	71/F	Cirrhosis	Susceptible to aztreonam, imipenem chloramphenicol, vancomycin, ofloxacin, and ciprofloxacin	Ciprofloxacin	Cured after amputation
Ferrer/1995 [17]	*M. odoratus*	Endocarditis	56/F	Chronic hemodialysis	Not available	Ceftazidime+netilmicin	Cured
Bachman/1996 [18]	*M. odoratus*	Cellulitis	63/M	COPD, chronic steroid use	Susceptible to trimethoprim-sulfamethoxazole, piperacillin, and imipenem	Imipenem then oral, trimethoprim/ sulfamethoxazole	Cured
Spanik/1998[12]	*M. odoratus*	Catheter	42/M	NHL	All strains except one were susceptible gentamicin,	Amikacin+ceftriaxone, catheter removal	Cured
			60/F	Gastric cancer	amikacin, ofloxacin,	Catheter removal	Cured
			31/M	AML	ciprofloxacin, netilmicin, tobramycin, azlocillin.	Catheter removal	Cured
			20/F	AML	Isolates from patient no. 4 were resistant to third-generation cephalosporins.	Catheter removal	Cured
Green/2001 [19]	*M. odoratus*	Cellulitis	69/M	HT, coronary angioplasty, recurrent cellulitis, trauma	Susceptible to piperacillin/ tazobactam, trimethoprim/ sulfamethoxazole, quinolones	Trimethoprim/ sulfamethoxazole	Cured
Motwani/2004 [20]	*M. odoratus*	Cellulitis	62/M	DM, peripheral vascular diseases	Susceptible to trimethoprim-sulfamethoxazole and quinolones	Trimethoprim/ sulfamethoxazole +ciprofloxacin	Cured
Bachmeyer/2008 [21]	*M. odoratimimus*	Cellulitis	49/M	Alcoholic cirrhosis	Susceptible to ciprofloxacin and rifampicin	İmipenem+ ciprofloxacin	Cured
Benedetti/2011 [22]	*M. odoratimimus*	Pneumonia, cellulitis	72/M	Trauma	Susceptible to piperacillin/ tazobactam, ticarcillin/ clavulanate and carbapenems	Piperacillin/tazobactam	Cured
Crum-Cianflone/ 2014 [13]	*M. odoratus*	Necrotizing fasciitis	55/F	Cirrhosis, morbid obesity, lymphedema	Susceptible to meropenem	İmipenem-cilastatin (intermediate susceptible)+ doxycycline	Died
Endicott-Yazdani/ 2015 [23]	*M. odoratimimus*	Diabetic foot ulcer	75/M	Diabetes, trauma	Not available	Meropenem	Cured

(continued)

Table 3. Continued

Reference/year	Species	Source	Age/ sex	Host factors	Resistance	Treatment	Outcome
Belloir/2016 [24]	*M. odoratimimus*	Erysipelas	92/F	Atrial fibrillation, CKD, HT, obesity	Susceptible to quinolones and carbapenems	Ciprofloxacin	Cured
Williems/2016 [25]	*M. odoratimimus*	Fulminant erysipelas	72/M	Steroid use, scratch by a domestic dog	Susceptible to levofloxacin, clindamycin, meropenem, tigecycline	Levofloxacin	Cured
Jover-Saenz/2016 [26]	*M. odoratimimus*	Prosthetic infection	76/M	Chronic hepatitis C, DM, knee prosthesis replacement surgery 17 years ago	Susceptible to piperacillin/ tazobactam, ciprofloxacin and minocycline	Ciprofloxacin +tigecycline then oral levofloxacin+ minocycline Arthrodesis	Cured
Beharrysingh/2017 [11]	*Myroides* spp.	Cellulitis	64/M	DM, Merkel cell carcinoma (a dose of carboplatine/ etoposide)	Susceptible to meropenem, trimethoprim/ sulfamethoxazole	Meropenem	Cured
LaVergne/2019 [1]	*M. injenensis*	Cellulitis	74/M	Alcoholic cirrhosis, Crohn's disease	Susceptible to ampicillin/ sulbactam, piperacillin/ tazobactam, quinolones and meropenem	Ampicillin/sulbactam	Cured
Meyer/2019 [27]	*Myroides* spp.	Cellulitis	69/M	COPD, HT, DM, trauma, use of chronic inhaled steroids, licked by domestic dog	Susceptible to piperacillin/ tazobactam, quinolones and imipenem	Ciprofloxacin	Cured
Pérez-Lazo/2020 [28]	*M. phaeus*	Catheter (dialysis)	57/F	HT, DM, CKD (RRT), multiple miyeloma (bortezomib)	Susceptible to piperacillin/ tazobactam	Piperacillin/tazobactam Catheter removal	Cured
Lu/2020 [3]	*M. odoratimimus*	Catheter	48/F	None	Susceptible to cefoperazone/ sulbactam	Cefoperazone/sulbactam Catheter removal	Cured
Foo/2020 [14]	*Myroides* spp.	Cellulitis	87/F	DM, HT	Not available	Meropenem	Died
Kurt/2021	*M. odoratimimus*	Catheter (central venous and dialysis)	64/M	Coronary by-pass surgery, COVID-19		Colistin+fosfomycin,+ trimethoprim/ sulfamethoxazole	Died

AML: Acute myeloid leukemia, CKD:Chronic kidney disease, COPD: Chronic obstructive pulmonary disease, DM: Diabetes mellitus, F:Female, M:Male NHL:Non-Hodgkin lymphoma, HT: Hypertension, RRT:Renal replacement therapy.

resistance genes associated with resistance to beta-lactams ($bla_{OXA-347}$, bla_{MUS-1}, bla_{EBR-1}), tetracyclines (*tetX*), sulfonamides (*sul2*), macrolides (*ereD*), (*ermF*) have been detected. Although other resistance genes could not be identified further analysis is required.

In conclusion, Myroides spp. are opportunistic environmental microorganisms and may lead to bacteremia secondary to soft tissue or catheter related infections especially in immunocompromised patients. Although generally susceptible to one or more classes of antibiotics, resistance genes to many classes of antibiotics and pan-resistant isolates have been reported and the infection may end with fatality.

AUTHOR DECLARATION

No conflict of interest in terms of financial and other relationships is present for any of the authors. The manuscript has been read and approved by all of the authors.

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