MYROIDES SP, A RARE OPPORTUNISTIC INFECTIVE AGENT, AT A HOSPITAL IN TURKEY

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Abstract. *Myroides* sp is a rare cause of infection, which can be fatal. *Myroides* spp isolates were obtained from urinal specimens of in- and out-patients attending a hospital in Turkey during July 2015 to November 2017. *Myroides* sp identification was based on colony morphology, biochemical properties and partial sequence of 16S rDNA, revealing the presence of *M. odoratus*. Antibiogram profiles showed almost all *Myroides* sp strains from in-patients (n = 11) were resistant to 13 antibiotics tested except for 50% that were intermediate resistant to tigecycline, whereas strains from out-patients (n = 4) were susceptible or intermediate susceptible. However, all *Myroides* sp strains lacked the six carbapenem resistance genes examined. Pulse-field gel-electrophoresis demonstrated clonality among four strains from in-patients. Clinical features of five in-patients and two out-patients isolates were believed to be due to *Myroides* infection and were treated accordingly; however, two died. Two out-patients believed to be infected recovered completely upon treatment. Ten in-patients had renal problems and all out-patients had urological problems or chronic renal failure. *Myroides* spp caused infection in both immunocompromised and immunocompetent patients in our study. Although tigecycline was used as first line treatment for *Myroides*-infected in-patients at this hospital, antibiograms of *Myroides* spp cultured from both in- and out-patients at other hospitals should be maintained to assist in prescribing appropriate antibiotics. Although *Myroides* infection is rare, its innate multi-drug resistance and propensity among patients with renal and urological problems warrants microbiological attention.

Keywords: *Myroides* sp, multi-drug resistance, renal pathology, Turkey

INTRODUCTION

Myroides is a gram-negative, aerobic, nonmotile, and nonfermentative bacillus (Cho *et al*, 2011). *Myroides* is usually found in soil, sea water, food and sewage plants (Sharma *et al*, 2016). *M. odoratus* and *M. odoratimimus* are the earliest discovered and best known *Myroides* spp (Schreckenberger *et al*, 2003), with *M. pelagicus*, *M. profundi*, *M. marinus* (Yoon *et al*, 2006; Zhang *et al*, 2008; Cho *et al*, 2011), *M. phaeus* (Yan *et al*, 2012), *M. xuanwuensis* (Zhang *et al*, 2014) and *M. indicus* (Beharrysingh,

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2017) subsequently isolated. *Myroides* sp forms pale yellow colored colonies due to the presence of flexirubin pigment, which are oxidase positive, and urea and indole negative. *Myroides* sp has a fruity smell (Hu *et al*, 2016; Sharma *et al*, 2016).

Myroides spp are not normal human flora and rarely infect humans (Sharma *et al*, 2016), but can be contracted from the environment (Benedetti *et al*, 2011). Although *Myroides* is considered a low-level pathogen (Elantamilan *et al*, 2015), it can cause meningitis, pneumonia, and urinary tract and soft tissue infections (Cho *et al*, 2011), which can become life-threatening in patients with immunodeficiency (Cho *et al*, 2011).

In this study, clinical presentation and outcome of *Myroides*-infected patients at Konya Numune Hospital, Konya, Turkey were investigated. *Myroides* sp was identified based on colony characteristics and 16S rDNA sequence. Clonality and antibiogram profile of the isolates were also investigated. The study should provide data important for diagnosis and treatment of this rare and often virulent bacterium.

MATERIALS AND METHODS

Study group

Records of all patients attending Konya Numune Hospital, Konya, Turkey from July 2015 to November 2017 with positive cultures for *Myroides* sp were reviewed. Demographic data, clinical characteristics, co-morbidities, treatment and outcome of both in- and out-patients were recorded.

The study was approved by the Ethics Committee of Konya Numune Hospital (20.12.2017/19). Participant consent was not required as this was a retrospective study.

Infection criteria and treatment outcome

A patient is considered to be infected with *Myroides* sp (positive urine culture) if presenting with fever, hypothermia or tachycardia together with urinary leukocytosis and elevated procalcitonin level, C-reactive protein or white blood cell count. Clinical improvement is defined as resolution of fever, tachycardia, pyuria or leukocytosis and reduction in procalcitonin level. Treatment failure is defined as a lack of clinical improvement evidenced by a lack of improvement in laboratory abnormalities and presence of infectious signs. Bacterial eradication is accepted when urine culture (performed 5-7 days after initiating treatment for in-patient and 10-14 days for out-patient) showed no *Myroides* sp growth.

Laboratory analysis

Urine samples were brought to the laboratory within 30 minutes of collection, incubated under aerobic conditions at 37°C for 24-48 hours and considered to be *Myroides* sp if there is no growth on eosin methylene blue agar, but growth on sheep blood agar (bioMérieux, Lyon, France) as 1-2 mm, round, smooth yellow-pigmented colonies (Elantamilan *et al*, 2015). Colonies were gram-negative, oxidase- and catalase-positive bacilli. VITEK 2 system (bioMérieux) was used for identification and testing of antibiotics susceptibility according to Clinical and Laboratory Standards Institute guidelines for non-Enterobacteriaceae (CLSI, 2014).

PCR amplification and sequencing of 16S rDNA fragment

DNA was extracted from isolates using a boiling method after being cultured for 18-24 hours on sheep blood agar (bioMérieux) at 37°C under aerobic condition (Dashti *et al*, 2009). A 1,500-bp 16S rDNA fragment was amplified using primers 27F

(5′-AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), where M = A/C and Y = C/T (Gutiérrez *et al*, 2012). The reaction mixture contained 4 μl of DNA, 1X DreamTaq Master Mix (Thermo Scientific, San Jose, CA) and nuclease-free water to make a 25-μl solution. Thermocycling was performed in a GeneAmp® PCR System 9700 instrument (Applied Biosystems, Foster City, CA) as follows: 95°C for 5 minutes; 40 cycles of 95°C for 45 seconds, 60°C for 45 seconds and 72°C for 78 seconds; and a final step of 72 °C for 10 minutes. Amplicons were purified using ExoSAP-IT™ (Thermo Fisher Scientific) and sequenced using a CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA) employing Dye Terminator Cycle Sequencing Kit (Beckman Coulter) and primer 787R (5′-GGACTACCAGGGTATCTAAT-3′) (Wang and Qian, 2009). Sequences were compared with those at GenBank database using Basic Local Alignment Search Tool (BLAST) and deposited as accession nos. MK508839 - MK508842.

Pulsed-field gel-electrophoresis (PFGE) typing

PFGE was performed as described previously (Morrison *et al*, 1999). In brief, DNA was digested with *Sma*I (Fermantas, Waltham, MA) and electrophoresis was performed in 1% pulsed-field certified agarose (Bio-Rad Lab, Hercules, CA) using a CHEF-DR III system (Bio-Rad Lab, Nazareth, Belgium) under the following conditions: 6 V/cm, 120° switch angle at 14°C, first block switch time of 3.5-25.0 seconds for 16 hours, second block switch time of 1-55 seconds for 6 hours, and a total running time of 22 hours. Gel then was stained with ethidium bromide (5 μg/ml), visualized under UV light and photographed using Gel Logic 2200 imaging system (Kodak, Rochester, NY). PFGE patterns were analyzed using BioNumerics software version 7.5 (Applied Maths, Saint-Matins-Latem, Belgium) and compared using a Dice coefficient with a tolerance of 1.5% and an optimization of 1%.

Multiplex PCR detection of carbapenem resistance genes

Multiplex PCR was performed using primer pairs specific to seven carbapenemase genes (Table 1). Reaction mixture (20 μl) contained 2.5 μl of 10X reaction buffer (Fermentas), 1.25 mM $MgCl_2$, 200 mM of each dNTP, 10 pmol of each seven primer pairs, 2.5 U Taq DNA polymerase (Fermentas) and 2 μl of DNA. Thermocycling was performed in GeneAmp® PCR System 9700 instrument (Applied Biosystems) as follows: 94°C for 5 minutes; 35 cycles of 95°C for 60 seconds, 60°C for 30 seconds and 72°C for 90 seconds; and a final step of 72°C for 10 minutes. Amplicons were separated by 1.5% agarose gel-electrophoresis and recorded as described above.

Statistical analysis

Data were evaluated using Statistical Package for the Social Sciences (SPSS), version 22 (IBM, Armonk, NY). Numerical data are expressed as mean ± SD and categorical data were as percentage. Comparisons between data of in- and out-patients were performed using chi-square or Fisher exact test where appropriate for categorical data and Mann-Whitney *U* test for numerical data. A p-value <0.05 is considered statistically significant.

RESULTS

Myroides spp were isolated from urine samples of 4 out-patients and 11 in-patients during the study period (Table 2). Sequences of the 1,500 bp 16S rDNA of four isolates had 99% similarity to *M. odoratus* (GenBank accession

Table 1
Primers used in multiplex PCR amplification of carbapenemase genes.

Target gene	Primer (5′ → 3′)	Amplicon size (bp)	Reference
OXA-23	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	501	Hou and Yang (2015)
OXA-48	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	733	Poirel *et al* (2011)
OXA-58	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599	Zhou *et al* (2007)
NDM	GTAGTGCTCAGTGTCGGCAT GGGCAGTCGCTTCCAACGGT	476	Mushtaq *et al* (2011)
VIM	GTGTTTGGTCGCATATCGC CGCAGCACCAGGATAGAAG	380	Garza-Ramos *et al* (2008)
KPC	ATGTCACTGTATCGCCGTC TTTTCAGAGCCTTACTGCCC	893	Gómez-Gil *et al* (2010)

Table 2
Number of cases with microbiological confirmed *Myroides* infections during the study period at Konya Numune Hospital, Konya, Turkey.

Patient	2015		2016	2017							
	Jul	Oct	Oct	Feb	May	Jun	Jul	Aug	Sep	Oct	Nov
In-patient	0	0	1	1	1	3	2	1	0	1	1
Out-patient	1	1	0	0	0	0	0	0	1	1	0

no. MK168622. Of four isolates analyzed by PFGE two clones were observed, one clone constituting strains from general intensive care unit (ICU) and the other from urology polyclinic and neurology ICU (Fig 1). The seven carbapenemase genes (encoding KPC, NDM-1, OXA-23, OXA-48, OXA-58, and VIM) were not detected (results not shown). All strains isolated from out-patients were susceptible or intermediate susceptible to all thirteen antibiotics tested, and all strains isolated from in-patients were resistant to all antibiotics but six strains were moderately susceptible to tigecycline (Table 3).

Mean age of in-patients (74 years) is significantly higher than out-patients (52 years) (Table 4). APACHE II and Charlson comorbidity scores of in-patients (all ICU patients) are significantly higher than those of out-patients.Ten in-patients had renal problems (acute renal failure or renal replacement therapy), and all out-patient subjects had urological problems (prostate hypertrophy or urinary stone disease) or chronic renal failure. Eight of the 11 in-patients and 1 of the 4 out-patients had a history of antibiotic use during the previous 30 days, with mean duration of antibiotic use of 17 and 3.5

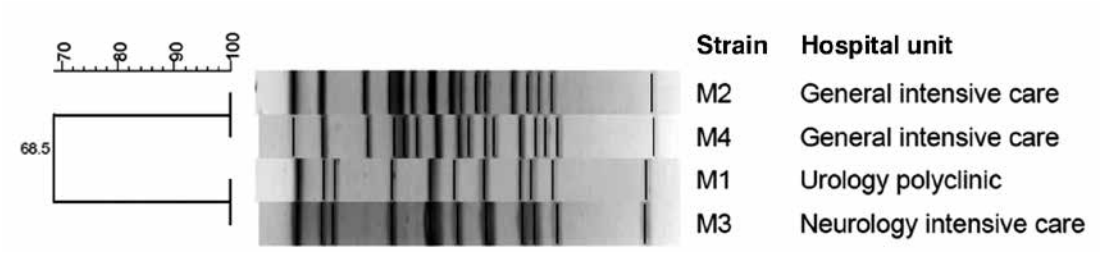

Fig 1-Pulsed-field gel-electrophoresis patterns of *Myroides* sp strains from in-patients admitted at Konya Numune Hospital, Konya, Turkey, from July 2015 to November 2017. DNA was digested with *Sma*I and electrophoresis was performed in 1% agarose using a CHEF-DR III system (Bio-Rad Lab, Nazareth, Belgium).

Table 3
Antibiogram of *Myroides* sp strains from patients at Konya Numune Hospital, Konya, Turkey from July 2015 to November 2017.

Antimicrobial	In-patient (n = 11)[a]				Out-patient (n = 4)[a]			
	Resistant	MIC (μg/ml)	Intermediate susceptible	MIC (μg/ml)	Susceptible	MIC (μg/ml)	Intermediate susceptible	MIC (μg/ml)
Colistin	10/10	>16			2/2	≤0.50		
Ciprofloxacin	11/11	>4			2/4	≤1	2/4	≤4
Cefepime	10/10	>32			2/2	≤0.12		
Imipenem	10/10	>16			2/4	≤0.25	2/4	≤2
Gentamicin	10/10	>16			2/4	≤1	2/4	≤8
Ceftazidime	10/10	>64			2/4	≤8	2/4	≤16
Meropenem	10/10	>16			2/4	≤0.25	2/4	≤16
Piperacillin-tazobactam	11/11	>128			3/4	≤4	1/4	≤16
Trimethoprim /sulfamethoxazole	11/11	>320			2/3	≤20		
Amikacin	11/11	>64			1/3	≤4	2/3	≤32
Tigecycline	4/10	>8	6/10	≤4	3/3	≤0.50		
Netilmicin	5/5	>32			1/3	≤1	2/3	≤16
Tobramycin	5/5	>16			1/3	≤2		

[a] For some antimicrobials tested, number of samples were less than total number of patients due to availability of drugs at time of test.

Table 4
Characteristics of patients with positive *Myroides* sp cultured at Konya Numune Hospital, Konya, Turkey from July 2015 to November 2017.

Characteristic	All patients Mean (±SD) (n = 15)	In-patients Mean (±SD) (n = 11)	Out-patients Mean (±SD) (n = 4)	p-value[a]
Age (years)	68 (16)	74 (11)	52 (20)	0.016
GKS	12 (4)	12 (4)	15 (0)	0.150
APACHE II score	20 (11)	25 (10)	9 (2)	0.011
Male, n (%)	5 (33)	4 (36)	1 (25)	0.680
Charlson comorbidity score	6 (3)	7 (2)	3 (2)	0.0001
Antibiotics use in previous 30 days, n (%)	9 (60)	8 (73)	1 (25)	0.095
Length of antibiotics use during previous 30 days (day)	14 (9)	17 (8)	4 (4)	0.070
Other previous infection in previous 30 days, n (%)	6 (40)	5 (46)	1 (25)	0.475
Time for growth in culture after admission (day)	-	23 (26)	-	
Long-term use of urinary catheter, n (%)	11 (73)	11 (100)	-	
Renal replacement therapy, n (%)	10 (67)	10 (91)	-	
Immune suppression or on steroid, n (%)	3 (20)	3 (27)	-	
Polymicrobial infection, n (%)	4 (27)	4 (36)	-	
Patients considered as infected and treated, n (%)	7 (47)	5 (46)	2 (50)	0.876
Clinical response in infected patients, n (%)	7 (47)	3/5 (60)	2/2 (100)	0.290
Microbiological eradication in infected patients, n (%)	3 (20)	2/5 (40)	1/2 (50)	0.846

[a] Compared between in- and out-patients. APACHE II, acute physiology and chronic health evaluation II; GKS, Glaskow coma score.

days in the former and latter group, respectively. Five and two of in- and out-patients presenting clinical signs and symptoms of infection received treatment (with ciprofloxacin-tigecycline, ertapenem or piperacillin-tazobactam), and three of the treated in-patients showed clinical improvement, with two microbiologically confirmed bacterial eradication. Both of the treated out-patients had clinical improvement, with one microbiologically proven bacterial eradication (Table 4).

Table 5
Clinical picture of in-patients with positive *Myroides* sp culture at Konya Numune Hospital, Konya, Turkey from July 2015 to November 2017.

Characteristic	Number of patients (%) (n = 11)
Duration of ICU stay in days, mean (± SD) day	36 (31)
Duration of hospital stay, mean (± SD) day	41 (35)
ICU ward	
Emergency	7 (64)
Clinical	1 (9)
Others	3 (2)
Outcome	
Died	5 (46)
Discharged	6 (55)
On mechanical ventilation	8 (73)
Duration of mechanical ventilation, mean (± SD) day	23 (32)
Underwent tracheostomy	3 (27)
With sepsis	7 (64)
Fitted with central venous catheter	8 (73)

Mean length of hospitalization was 41 days and of the 5 in-patients with clinical presentations of infection, 3 recovered and 2 died (Table 5).

DISCUSSION

Although *Myroides* infection is considered to be acquired from the environment (Benedetti *et al*, 2011), it can also be nosocomial (Hu *et al*, 2016). Hugo *et al* (2006) proposed water used in hospitals might be a source of infection and it has also been reported to occur from nosocomial transmission (Benedetti *et al*, 2011). Several studies reported such *Myroides* outbreaks might have originated from operating rooms or ICUs but the actual source remains unknown (Benedetti *et al*, 2011; Ktari *et al*, 2012).

Ktari *et al* (2012) reported a nosocomial *Myroides* urinary tract infection among 7 patients in a Tunisian hospital. In our study, all in-patients in ICU had *Myroides* urinary tract infection, the first report from Turkey. Two strains of the same clonality were found in the same ICU and another two strains originating from a different clonal origin were isolated from two different hospital care units.

Myroides spp are usually low-grade opportunistic pathogens (Beharrysingh, 2017), often occurring in immunosuppressed patients with diabetes or chronic obstructive pulmonary disease, and those undergoing corticosteroid treatment (Beharrysingh, 2017), with alcoholism, diabetes mellitus, malnutrition, prematurity, malignancy and immunosuppression (Benedetti *et al*, 2011).In our study, Charl-

son comorbidity and APACHE II scores were not related to *Myroides* infection but to seriousness of illness.

Yagci *et al* (2000) reported among 13 *Myroides* cases, four have urinary neoplasm and nine with urinary stones. Among our 11 in-patients, 10 and all out-patients had urological or renal problems, similar to the findings from Tunisia (Ktari *et al*, 2012). A previous study of endocarditis cases due to *Myroides* also had end stage renal disease (Ferrer *et al*, 1995).

Myroides spp isolated from in-patients were highly resistant to thirteen antibiotics but 54% were intermediate susceptible to tigecycline, but the cause for this phenomenon is not fully understood (Hu *et al*, 2016). *Myroides* spp have been reported to be resistant to β-lactams, monobactams, carbapenems, and aminoglycosides (Maraki *et al*, 2012), with resistance to β-lactams due to production of chromosome-encoded metallo-β-lactamases (TUS-1 and MUS-1) (Mammeri *et al*, 2002). Clinical improvement was seen in three patients treated with ciprofloxacin-tigecycline, ertapenem and piperacillin-tazobactam, respectively, but there was a fatality in one patient who did not respond to the latter combination drug treatment. There are previous reports of clinical and microbiological cure of *Myroides* infection in a pregnant patient (Elantamilan *et al*, 2015), in endocarditis treated with meropenem (Sharma *et al*, 2016) and in a patient receiving chemotherapy and treated with meropenem (Beharrysingh *et al*, 2017).

On the other hand, all *Myroides* spp isolated from out-patients were susceptible or intermediate susceptible to the tested antibiotics. Two out-patients considered to be infected with *Myroides* were successfully treated with cefepim-nitrofurantoin and cefaclor, respectively. Ktari *et al* (2012) reported, prior to isolating *Myroides* spp, suspected infected patients are treated with a number of antibiotics, especially carbapenems.

In summary, the majority of patients attending a hospital in Turkey, from whom *Myroides* spp were isolated, have urological or renal problems. *Myroides* strains from in-patients were multi-drug resistant with only 50% being intermediate sensitive to tygecycline. Thus, *Myroides* should be diagnosed in patients with urinary tract infection and a history of urological or renal disease that do not respond to regular antibiotics treatment. Antibiogram profile should be determined of *Myroides* spp to assist in the proper choice of antibiotics. Further studies are needed to identify etiologic factors associated with *Myroides* infection to provide effective control and prevention measures of this rare, but potentially fatal, bacterial infection.

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