Nosocomial infection of extensively drug-resistant *Myroides odoratimimus* in a Turkish hospital

Introduction

Myroides spp., which was isolated from the human intestine in 1923 for the first time, was classified as *Flavobacterium* spp., and gram negative, aerobic, lactose nonfermentive, oxidase-positive and non-motile bacteria with yellow pigment (positivity of flexirubin).[1-3] Due to important genomic and phenotypic characteristics, *Myroides* spp. were reclassified as a new genus.[4,5]

The genus *Myroides* originally included two species: *Myroidesodoratus* and *Myroides odoratimimus.* These are environmental microorganisms seen in soil and water, but not a part of human microflora.[6] *Myroides injenensis* was first isolated from human urine in 2012.[7,8]

M. profundi, *M. marinus*, *M. phaeus*, *M. pelagicus*, *M. guanonsis*, and *M. xuanwensis*are the other species in this family, but are not associated with human infections.[8-10] *Myroides* spp. can also be isolated from meat-processing plants, the gut of adult flesh flies and seafood products.[11-13]

Myroides infections are mostly reported inimmunocompromised individuals, they rarely cause disease in non-immunocompromised individuals.[3,14] *Myroides* genus can also cause community or hospital infections as they are low-grade opportunistic pathogens. In addition, *Myroides* spp. can cause soft tissue infections, septic shock, pneumonia, systemic infections, necrotizing fasciitis, urinary tract infections (UTI) or erysipelas.[15] *M. odoratimimus* causes outbreaks of life-threatening urinary tract infections due to its multiple drug resistance and unknown pathogenicity.[16]

To date, outbreaks of hospital-acquired infections caused by Myroides ssp. have been rarely reported in the literature.[15,17-19]

In this study, we reported a nosocomial outbreak due to *M. odoratimimus* causing UTI in a hospital in Turkey. To our knowledge, this is the third outbreak recorded in Turkey and the fifth outbreak reported in the literature. In addition, this is the largest outbreak in the literature.

Materials and methods

Between October 2018 and December 2019, a total of 25 *M. odoratimimus* isolates were included in the study, isolated from samples of 20 patients who were admitted to a hospital in Turkey. The study was approved by the local ethics committee and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from the patient or relatives.

Bacterial Identification

All samples were inoculated onto 5% sheep blood agar, eosin methylene blue agar and chocolate agar media for initial identification and incubated at 37 °C under aerobic and microaerophilic conditions for 18-24 hours. After incubation, all of the isolates were first stained with gram stain and examined under a microscope and then an oxidase test was applied to the isolates detected as gram negative bacillus. Further phenotypic and genotypic identification of gram negative and oxidase positive bacilli were performed using three different methods; VITEK®-2 automated identification system, Matrix Assisted Laser Desorption/Ionisation-Time of Flight Mass (MALDI-TOF MS) Spectrometry with MALDI Biotyper (BrukerDaltonics, Germany) Identification and 16S-RNA Microbial Diagnosis methods.

Antibiotic Susceptibility Test Procedure

VITEK®-2 GN AST-N222 (BioMérieux, France) cards were used for antibiotic susceptibility tests and the cards were loaded into the VITEK®-2 (BioMérieux, France) automated system according to the manufacturer's recommendations. The fifteen antibiotics tested were: amikacin, aztreonam, ceftazidime, ciprofloxacin, cefepime, gentamicin, imipenem, levofloxacin, meropenem, netilmicin, piperacillin, trimethoprim/sulfamethoxazole, tetracycline, tobramycin and piperacillin/tazobactam with VITEK®-2 (BioMérieux, France) automated system according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) standards.

MALDI-TOF Spectrometry Identification System

Using the VITEK®-2 (BioMérieux, France) automated system, isolates identified as *Myroides* spp. with a probability of ≥95% were subsequently redefined using the MALDI-TOF mass spectrometry system (Bruker Daltonics, Bremen, Germany).

16S-RNA Microbial Diagnosis

16S-RNA Microbial Diagnosis (DNA isolation + PCR + DNA Sequence analysis + Bioinformatic Analysis) was performed for three isolates. DNA extraction was performed on freshly-harvested bacterial colonies using the Qiagen Blood and Tissue Isolation Kit (Hilden, Germany) according to the instructions of the manufacturer. For detection of *Myroides* spp. 16S-RNA, the primers 907R ' CCGTCAATTCMTTTGAGTTT 3' 27F 5' AGAGTTTGATCMTGGCTCAG 3 were used in a touch-down PCR assay. DNA sequencing was performed on the ABI 3100 Genetic Analyzer using the Bigdye Cycle Sequencing kit v.3.1 kit.

Epidemiological Analysis

Environmental surveillance sampling

Environmental surveillance samples taken from the patients' clinics included urine bags, urine bag taps and urine bin, wet wipes used for perineum cleaning, area of urinary culture containers, cabinets forsterile gauze/pads, clean laundry cart, care pads, sink hand antiseptic solutions, liquid soap, taps and tap water etc.

Repetitive Extragenic Palindromic Elements (REP) PCR Assay

GeneMATRIX Tissue & Bacterial DNA Purification KIT (EURx Ltd., Gdańsk, Poland) was used for DNA extraction. The obtained DNA was stored at-20 °C until the analysis was done. The REP-PCR was conducted using the methods and primers (REP1R-I (5'-IIIICGICGICATCIGGC-3'), REP2-I (5'- ICGICTTATCIGGCCTAC-3')) developed by Reboli et al.[20] *Acinetobacter baumannii* strain was used as a control to show that the REP-PCR method can make good DNA typing in different epidemiological groups.

Statistical analysis

The data are expressed as mean+standard deviation, median and percentage values using MedCalc statistical software demo version 17.6 (Ostend, Belgium, 2017).

Results

The mean age of the patients was 69.85±15.57 years (range 24-88). Twelve of 20 patients were male and 8 were female. One of the patients was hospitalized in the surgical clinic, one in chest diseases clinic and the other 18 were in the 2nd and 3rd level intensive care units. Four of the patients had chronic obstructive pulmonary disease (COPD) or pneumonia, and two had cardiac arrest due to these diseases, were successfully resuscitated and then taken to the intensive care unit. Two patients were connected to a mechanical ventilator after the operation due to acute subdural hematoma, and two of them were in the intensive care unit due to ischemic cerebrovascular disease. One patient was hospitalized for incisional hernia operation, one for hip prosthesis operation and one patient for acute pancreatitis. All patients had uretero-vesicular catheterization to monitor urine flow. Patients other than those with COPD or pneumonia were catheterized at first admission to the emergency room. All patients, except one with nosocomial bacteraemia, had nosocomial urinary tract infections. Table 1 shows the age, gender, hospitalisation clinics, hospital admission date, comorbidities and indwelling device of the patients and date of samples.

The average hospitalisation time before isolation of these pathogens was 75.4 ± 96.6 days (range 16-480). One patient (C1) who was receiving meropenem and tigecycline therapy was completely cured. It was found that good clinical response was obtained in patients who received tigecycline (C7) or combined therapy with tigecycline and meropenem (C9) or ceftazidime, colistin and tigecycline (C12) or meropenem and piperacillin-tazobactam (C13). Ten patients died. Hospitalisation time, types of infections, antibiotics used, outcome and concomitant infections of patients are shown in Table 2.

None of the patients with *M. odoratimimus* isolated had systemic corticosteroid usage or solid organ malignancy. However, 6 patients diagnosed with diabetes mellitus were considered immunosuppressive.

Infection markers of the patients were as follows; procalcitonin mean 4.16 ± 4.19 ng/ml (range 0.14-15 ng/ml), WBC count mean 8998±2978/ml (range 3720-15310/ml), neutrophil count mean 6859 ± 2481/ml (range 1822-11757), mean platelet count 197450 ± 111625/ ml (range 87000-493000 / ml) and mean CRP 133.85 ± 52.74 mg/L (range 32-227 mg/L). Immunosuppression states and infection markers of patients are shown in Table 3.

The antimicrobial sensitivity of *M. odoratimimus* isolates were evaluated. One isolate was sensitive to piperacillin/tazobactam (MIC: ≤4 µg/ml) and one isolate was moderately sensitive to cefepime (MIC: 16 µg/ml). Other *M. odoratimimus* isolates were resistant to beta-lactams (including extended spectrum cephalosporins and beta-lactamase inhibitors), monobactams, carbapenems, aminoglycosides, fluoroquinolones, and sulphonamides. MIC values of the antimicrobial agents detected by VITEK 2 are shown in Table 4.

The first *Myroides* spp. identification in our hospital was made in November 2018, the time interval from October, November and December 2019 had highest most isolationof *M. odoratimimus* species with 11 isolates. Distribution of *M. odoratimimus* isolates by months is shown in Table 5. *Myroides* spp. could not be detectedin environmental samples.

All isolates were identified as *M. odoratimimus* by MALDI TOF MS and three isolates were identified as *M. odoratimimus* by 16S rRNA. Raw sequencing data is available from NCBI in SUB7440670 Seq1 MT465451, SUB7440670 Seq2 MT465452, SUB7440670 Seq3 MT465453.

Analysis of REP PCR patterns

The last ten isolates were evaluated by Repetitive Extragenic Palindromic Elements (REP) PCR. When the similarities of DNA fingerprints were visually examined, there was no heterogeneity in the density and shape of the bands. The DNA patterns of the isolates were similar, the missing band was not seen, and all the visible bands of all isolates had the same migration distance. In this study, similar DNA patterns of 10 isolates typed by REP-PCR are shown in Figure 1.

DISCUSSION

Nowadays, the spectrum of community-based and hospital-acquired infections caused by atypical pathogens are constantly being updated. There has been an increase in the number of newly described microorganisms due to the use of molecular methods such as 16SrRNA sequencing and MALDI-TOF mass spectrometry in clinical microbiology laboratories.[21]

Myroides spp., which is generally found in external environments such as soil and water, often causes various opportunistic infections in immunocompromised patients with history of

organ transplantation, solid malignancy, rheumatologic/inflammatory diseases, neutropenia, HIV diseases, diabetes mellitus and immunosuppressive therapy.[22-25] In our patient group, six patients had diabetes mellitus.

Myroides spp. can be classified as a multi-drug resistant environmental organism and can accommodate different resistance patterns simultaneously, as shown in this article and in other studies.[26] Intrinsic resistance to β-lactamases is due to the presence of two metallo-β-lactamases, MUS-1 and TUS-1, which share 73% amino acid identity.[27] Also, a resistance gene island was found on the chromosome of the bacterium [10]. This region contains different types of resistance genes such as text (conferring tetracycline resistance), cat (chloramphenicol resistance), bla-OXA-347 and bla-OXA-209 (conferring β-lactam resistance).[28]

Combined use of tigecycline[15] and quinolones with rifampicin[17] for the treatment of *M. odoratimimus* was reported to provide successful results. In the treatment of infections caused by other *Myroides* spp., there are articles reporting that the use of cotrimoxazole, meropenem, or piperacillin/tazobactam constitute a good clinical response.[2,14,29]

All *M. odoratimimus* isolates we detected in our study were resistant to almost all antimicrobials tested. In our study, a patient receiving meropenem and tigecycline treatment was successfully treated. Four patients who received tigecycline, meropenem, piperacillin-tazobactam, colistin and tigecycline responded well to the treatment. Follow-up of 5 patients continued in intensive care unit and 10 patients died. In addition to *Myroides* infections, we think that the reason for these patients' deaths are the contribution of primary diseases, mechanical ventilation, septicaemia, and pneumonia.

Antimicrobial resistance has reached alarming levels in different parts of the world. As a result of inappropriate and often uncontrolled use of antibiotics, many treatment options are ineffective.

In our hospital, pan-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and multidrug-resistant *Pseudomonas aeruginosa* infections have increased recently, and the use of antibiotics such as third-generation cephalosporin, carbapenem, colistin and linezolid has increased for the treatment of these infections. Therefore, we think that the widespread use of these broad-spectrum antibiotics may have contributed to the selection of *M. odoratimimus* isolates.

Also, *M. odoratimimus* isolates are a strong biofilm producer. Biofilms are sessile bacterial communities that adhere to both biotic and abiotic surfaces, such as medical devices. The bacteria are trapped in an extracellular polymeric matrix that they produce.[30] The production of a strong biofilm is a serious problem because it increases pathogenicity in device-related infections and it is often associated with therapeutic failure, as well as persistence of infections.[31] In the current study, all patients had a urinary catheter and we evaluated this as a predisposing factor for *M. odoratimimus* infections.

Holmes et al. identified six isolates of *Flavobacterium odoratum* from urine and three isolates from infected wounds, but they didn’t consider their strains actedas pathogens.[32] Until today,

M. odoratimimus was identified as the causative pathogen in UTI in only a few nosocomial outbreaks.[15,17-19]

In this study, we thought that 10 isolates typed by REP-PCR came from the same epidemiological origin because of similar DNA patterns and clusters of cases in the same period may be due to a nosocomial outbreak (Figure 1). In addition, this study is the first study in the literature where *Myroides spp.* isolates were investigated using REP-PCR.

Yağcı et al.[18] reported an outbreak of urinary tract infections caused by *F. odoratum*at a hospital in Turkey. They reported that all infected patients were catheterized or had urinary tract neoplasia or urinary calculi. In their report, two of the four patients had urological comorbidities (transurethral resection of the prostate and bladder cancer cystectomy with bilateral cutaneous ureterostomy for benign prostatic hyperplasia).[18]

Ktari et al.[17] reported 7 cases of UTIs originating from *M. odoratimimus* who had undergone endourological surgeries in a Tunisian hospital urology unit in 2012 and had urinary calculi (except one). Although the histories of urinary calculi, neoplasia and endourological surgery were common in both outbreaks, these conditions were not present in our patients.

Licker et al.[15] also reported a nosocomial outbreak in 4 immunocompromised patients in Romania in 2017. Risk factors for *Myroides* spp. infection in our patient group may be immune suppression, long-term hospitalisation (especially in intensive care), multiple drug resistance due to intensive antibiotic use, concomitant infections and urethro-vesicular catheterization.

Finally, Kutlu et al.[19] reported that *M. odoratimimus* strains were isolated from immunocompromised 6 patients in Turkey in 2020. All patients had urethral-vesical catheterization with a Foley's catheter.

As previously reported, the source of nosocomial *Myroidesspp* infections is unknown, although the transmission of bacteria in the hospital environment is suspected .[15,17-19]

The increase in *Myroides* infections, which started in November 2018 in our hospital, decreased with the review of asepsis and antisepsis procedures and the improvement of general infection control and prevention procedures, but it is not completely over.

Although comprehensive surveillance screening was performed in the departments where patients with *M. odoratimimus* were hospitalised in our hospital, the source of the pathogen could not be shown. While the increase in the number of urinary tract infections caused by this unusual organism occurred over a period of 13 months, it probably suggests a common source.

Since the source of endogenous infection could not be determined in these patients, it was thought that the bacteria might have been acquired from an environmental source. The patients' hospitalisation periods of at least two weeks and the presence of urinary catheters in all patients suggest that the antisepsis rules were not followed adequately in the emergency room or when the urine catheter was inserted in the clinics or during catheter maintenance.

We recommend that if urinary catheterisation will be performed for a long time, use of silicone catheters or antimicrobial coated catheters that are more resistant to chemical effects than latex catheters should be preferred.

There are some limitations to the current study. This study covers a limited number of patients and only one health centre which limits results. The rarely considered pathogenic *Myroides spp.* concomitant status in all our patients and long-term hospitalisation history in intensive care units can make the pathogenicity of *Myroides spp.* controversial alone. Further studies are needed in this regard.

Conclusions

Our study showed that although *M. odoratimimus* is a rare pathogen isolated from clinical specimens, it can lead to prolonged urinary tract infection in patients in hospital and especially in intensive care units and nosocomial urinary tract infections in immunocompromised patients.

It should be kept in mind that *Myroides spp.* isolates with multiple and broad-spectrum drug resistance may be a serious nosocomial pathogen such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii.* In order to choose the best treatment regimen, this atypical pathogen needs to be quickly identified and antibiotic susceptibility tests performed.