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Received: January 30, 2020.  Accepted: May 12, 2020.
Published online: May 29, 2020.
DOI: 10.7883/yoken.JJID.2019.536
A healthcare-associated outbreak of urinary tract infections due to *Myroides odoratimimus*

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**Running Title:** An outbreak due to *Myroides odoratimimus*

**Keywords:** *Myroides odoratimimus*, urinary tract infection, pulsed-field gel electrophoresis, nosocomial, outbreak
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Summary

*Myroides* spp. are low-grade opportunistic pathogens. There were only a few outbreaks due to *Myroides* spp. described in the literature to date. We report a healthcare–associated outbreak of urinary tract infections caused by *Myroides odoratimimus* in a Turkish hospital. From March to May 2019, six strains of *M. odoratimimus* were isolated from the urine samples of patients hospitalized in the intensive care units (ICUs). After identification and antibiotic susceptibility testing with VITEK 2 system, MALDI-TOF-MS and 16S rRNA based sequencing methods were performed for confirmation and species level identification. Pulsed-field gel electrophoresis (PFGE) was used to investigate clonal relatedness of the isolates. All the patients were immunocompromised and underwent urinary catheterization. None of them had urinary neoplasm, surgery or calculi. VITEK 2 and MALDI-TOF-MS systems revealed that the isolates belong to the *Myroides* genus but lacked to identify the isolates at the species level. 16S rRNA based sequencing successfully identified all the isolates as *M. odoratimimus*. The isolates were resistant to all antibiotics tested. All isolates had indistinguishable PFGE pattern indicating cross-transmission between cases. Although *M. odoratimimus* is rarely isolated from human specimens, clinicians should be aware of its ability to cause UTIs and outbreaks.

Introduction

*Myroides* spp., which are environmental bacteria isolated from soil and water, rarely cause disease in humans. The genus known as *Myroides* was first described as *Bacterium faecale aromaticum* and later renamed as *Flavobacterium odoratum*. After the identification of their unique characteristics such as halo-tolerance, gliding motility, abundant growth at 37°C, and
differences in fatty acid composition, they were reclassified in a new genus, *Myroides*, in 1996 (1-3). Based on DNA homology, carbon source assimilation profiles, and cellular fatty acid composition, firstly, *Myroides* genus was divided into the two species *M. odoratus* and *M. odoratimimus* (4) and then additional studies defined new species. Among the species, *M. odoratus*, *M. odoratimimus*, and *M. injenensis* can cause human infections, however, other *Myroides* species including *M. profundi*, *M. marinus*, *M. phaeus*, *M. pelagicus*, *M. guanonsis*, and *M. xuanwensis* were not associated with human infections until now (2,5). The *Myroides* species are Gram-negative, aerobic, nonmotile, asaccharolytic, urease, and oxidase-positive, rod-shaped bacteria. On nutrient agar, the colonies of *Myroides* species are yellow due to flexirubin production and have a fruity odor (3).

*Myroides* species were previously described as not highly pathogenic. However, nowadays these bacteria can be isolated from human infections with significant morbidity and mortality due to their multi-drug resistance, biofilm formation capacity and polysaccharide capsules (3, 6). These opportunistic pathogens can be responsible for soft tissue infections, septic shock and pneumonia, ventriculitis, systemic infections, tricuspid valve endocarditis, indwelling catheter infections, bacteremia, canaliculitis, necrotizing pancreatitis, prosthetic joint infections and urinary tract infections in immunocompromised patients and can cause life-threatening infections in immunocompetent hosts (2, 7, 8).

In literature, hospital-acquired outbreaks due to *Myroides* spp. have rarely been reported (7, 9, 10). Here, we reported a nosocomial outbreak due to *M. odoratimimus* causing urinary tract infections in a Turkish hospital, which is, to our knowledge, the second outbreak recorded in Turkey and the fourth described in literature to date. We investigated the clonal relationship between the isolates and compared the performance of VITEK² system (BioMérieux, Marcy l’Etoile, France) and matrix-assisted laser desorption/ionization-time of flight mass
spectrometry (MALDI-TOF-MS, bioMérieux, Nürtingen, Germany) system with the 16S rRNA based sequencing method.

Material and Methods

Patients and Isolates

Seven *Myroides* spp. isolates were recovered from urine samples of six patients in Usak Training and Research Hospital between March and May 2019. One of the patients had two urine samples, only the first isolate of this patient was included in the study. Six isolates recovered from different patients were included in the study.

The medical records of all patients with positive *Myroides* spp. urine cultures were collected retrospectively. The demographic data including comorbidities, use of an indwelling catheter, the antimicrobial treatment, and the clinical outcomes were reviewed.

Infections and colonization with multidrug-resistant organisms detected >48 hours after hospital admission were considered as healthcare-associated infections (HAI), according to the criteria of the Centers for Disease Control and Prevention (CDC) (11,12).

All experiments were carried out in compliance with the relevant laws and guidelines, in accordance with the ethical standards of the Declaration of Helsinki. The study was approved by the Usak University Faculty of Medicine Local Ethics Committee (No. 218-03).

Identification and Antimicrobial Susceptibility Testing

All isolates were identified as *Myroides* spp. by using the VITEK-2 system and MALDI-TOF-MS system. For identification of bacteria at the species level, 16S rRNA based sequencing was performed.
The 16S rRNA based sequencing

All *Myroides* isolates were cultured on sheep blood agar medium. After 48 h incubation at 35°C, bacterial inoculum was prepared in sterile phosphate buffer saline (PBS, pH 7.6) for each isolate by adjusting the turbidity to that of a 4 McFarland turbidity standard. Bacterial DNA was extracted by using GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Carlsbad, California) according to the manufacturer’s instructions (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012663_GeneJET_Genomic_DNA_Purification_Kit_UG.pdf). In brief, 400 µL Lysis Solution and 20 µL Proteinase K were added to 200 µL bacterial suspension and incubated at 56°C for 10 minutes. Then 200 µL absolute ethanol was added, vortexed and loaded into purification column inserted in a collection tube. The tube was centrifuged for 1 minute at 6000 x g and the column was washed two times with 500 µL of Wash solutions I and II for 60 seconds and 3 minutes, respectively. Lastly, the washed product was eluted into a new microcentrifuge tube with 50 µL Elution Buffer by centrifugation at 8000 x g for 1 minute. Two PCR assays were performed to amplify the 16S rRNA gene by using the primer pairs 27F (agagttgtatymtggctcag) / 878R (ggagtaccagggtatctaat), and 533F (gtgccagcmgccgcggtaa / 1492R (ggttaccttgttacgactt). PCR products were purified by using ExoSAP-IT™ PCR Product Cleanup Reagent (Affymetrix, Santa Clara, USA) according to manufacturer’s instructions and DNA concentrations were measured by NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). About 20 ng template DNA was used in four different sequencing reactions, each including 5 pmol of each primer, 4 µL BigDye Terminator 3.1 Ready Reaction Mix in a total of 10 µL reaction volume. After cycle sequencing, all products were purified by using BigDye X Terminator Purification Kit (Life Technologies, Carlsbad, USA) according to manufacturer’s instructions, then all samples were sequenced by ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA) instrument. DNA sequences of all isolates were submitted to
the National Center for Biotechnology Information (NCBI) and the *M. odoratimimus* species were identified according to 100% sequence identity with the strains in NCBI.

**Antimicrobial susceptibility tests (ASTs)**

ASTs were performed using the VITEK 2 system. The following antimicrobial agents were tested: piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem, gentamicin, amikacin, ciprofloxacin, levofloxacin, and colistin. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) on Antimicrobial Susceptibility Testing Criteria for other non-Enterobacteriaceae (13).

**Environmental Sampling**

After informing the Hospital Infection Control Committee, environmental surveillance samples from two adjacent intensive care units (ICU) (Neurology ICU, Respiratory ICU) and healthcare workers’ hand-swab samples were obtained and investigated for the presence of *Myroides* spp.

**Pulsed-field gel electrophoresis (PFGE) analysis**

The clonal relationship of isolates was analyzed by pulsed-field gel electrophoresis (PFGE) following the protocol optimized by Durmaz R. *et al* (14) with some modification. Briefly, *Myroides* isolates growing on sheep blood agar medium were used to prepare cell suspension equal to McFarland 4 turbidity in Cell Suspension Buffer [CSB: 100 mM Tris-HCL (pH 8), 10 mM EDTA]. Then 2% low melting point agarose (Gibco BRL, Paisley, United Kingdom) containing sodium dodecyl sulfate (SDS) with a final concentration of 1% was prepared. An equal volume of cell suspension and low-melting agarose were mixed, 100 µL of this mixture was dispensed into plug molds. The cells in plugs were lysed in Cell Lysis Solution 1 [CLS 1: 50 mmol/L Tris-HCl, 50 mmol/L EDTA, lysozyme (2.5 mg/mL), proteinase K (1.5 mg/mL), pH 8.0] and then Cell Lysis Solution 2 [CLS 2: 0.5 mol/L EDTA (pH 8.0), 1% sarcosyl, and proteinase K (400 mg/mL)]. The plugs were washed three times with sterile ultrapure water and
additionally three times with TE buffer (10 mmol/L Tris-HCl, 0.1 mmol/L EDTA, pH 7.6). As recommended by Ktari et al (9), bacterial DNA in plug was restricted with 2 μL SmaI fastDigest restriction endonuclease enzyme (Thermo Fisher Scientific). Fragmented DNA was electrophoresed in 1% pulsed-field certified agarose (Bio-Rad Laboratories, Hercules, CA) by using the CHEF-DR III system (Bio-Rad Laboratories, Nazareth, Belgium). Electrophoresis conditions were 14°C at 6 V/cm² for 20 hours. The initial and final switching times were 5 seconds and 30 seconds, respectively. The gel was stained with ultraviolet (UV) visible dye and visualized under UV light.

Results

Patients and Isolates

During two months (March to May 2019) six Myroides strains were isolated from the patients. All of the isolates were recovered from the patients hospitalized in ICUs. Out of six isolates, two isolates were recovered from Surgical ICU, and Internal Medicine ICU in March 2019. The other four isolates were isolated from two adjacent ICUs (Neurology ICU and Respiratory ICU) consecutively in two weeks in mid-May 2019.

Environmental samples taken from two adjacent intensive care units (Neurology ICU and Respiratory ICU) and healthcare workers’ hand-swab samples were negative for the presence of Myroides spp.

Demographic characteristics and clinical data of the patients

Demographic characteristics and clinical data of the patients are shown in Table 1. Four patients (67%) were men and two patients (33%) were women. The mean age of the patients was 73 years (range 54-85). All the patients underwent urethro-vesical catheterization with a Foley’s catheter. One patient (P3) had chronic obstructive pulmonary disease and asthma, for which he
received a long-term corticosteroid therapy. The other one (P2) had end-stage renal disease. All the patients had diabetes mellitus. None of them had a history of urinary surgery or urinary calculi.

Three (50%) of the patients (P1, P2, P5) were evaluated as urinary tract infections caused by *Myroides* species. The other patients were considered as bladder colonization (P3, P4, P6). Patient P1 was successfully treated with piperacillin/tazobactam. Patient P2 had ventilator-associated pneumonia and blood-stream infection due to carbapenem-resistant *Acinetobacter baumannii* and died from sepsis. Patient P5 was admitted to Respiratory ICU with pneumonia for which she received colistin-meropenem combination therapy and received fluconazole for the treatment of candiduria. After antibiotic therapy, samples for tracheal aspirate culture, blood culture, and urine culture showed no pathogenic bacterial growth. Due to elevation in body temperature and C-reactive protein (CRP) level, new culture samples were taken and the urine culture revealed *Myroides* spp. growth greater than 100,000 colony forming unit (cfu)/mL. The patient had other comorbidities and despite the treatment with tigecycline and fosfomycin, she died on the fourth day of *Myroides* isolation. These three cases were evaluated as HAI and *Myroides* species were isolated from those patients at a mean duration of hospitalization of 24 days (range 19-33).

**Identification and Antimicrobial Susceptibility Testing**

The isolates had a fruity smell and yellow pigment production on agar plates. By using, the VITEK®2 GN ID cards (BioMérieux, Marcy l’Etoile, France), all the isolates were identified as *Myroides* spp. By MALDI-TOF MS, all of the isolates were also identified in *Myroides* genus but species could not be identified by this method. However, 16S rRNA sequencing of the 1361 bp region revealed that all the isolates had identical DNA sequences with *Myroides odoratimimus* in the NCBI database. GenBank accession numbers of the stains were MN378638 (P1), MN378639 (P2), MN378640 (P3), MN378641 (P4), MN378642 (P5), and...
MN378643 (P6). A phylogram was generated by using DNA sequencing of the six *M. odoratimimus* strains identified in the current study and the strains in NCBI (Fig. 1).

Antibiotic susceptibility testing and determination of MICs revealed that all isolates were resistant to all antibiotics tested, including beta-lactams, monobactams, carbapenems, fluoroquinolones, aminoglycosides and polymyxins.

**Pulsed-field gel electrophoresis (PFGE) analysis**

The PFGE results of *M. odoratimimus* isolates revealed that all isolates had an indistinguishable pattern indicating that all isolates were originated from a common source (Fig. 2).

**Discussion**

Although *Myroides* species are known as environmental microorganisms, they can be rarely isolated from a variety of clinical samples such as urine, wound, sputum, blood, and ear discharge of human infections (2, 3). *Myroides odoratimimus* and *Myroides odoratus* are the most common *Myroides* species isolated from human infections. In addition to this, in literature one UTI and one cellulitis cases caused by *M. injenensis* were reported (2, 15). There were only a few outbreaks due to *Myroides* spp. described in the literature to date and all of them were urinary tract infections caused by *M. odoratimimus* (7, 9, 10). The first outbreak was described by Yağcı et al. (10), over three years in a Turkish hospital, all the patients who developed *Myroides* spp. urinary tract infections had urinary calculi or urinary neoplasms. The second outbreak was reported in the urology department of a Tunisian hospital in 2012 (9). In this outbreak, all patients but one had urinary calculi and underwent endourological surgery and all isolates were resistant to all antibiotics tested. Three patients had been successfully treated with ciprofloxacin and rifampicin (9). The third outbreak, which occurred in a Romanian hospital, reported four cases of UTIs due to *M. odoratimimus* from June to August 2017. Three of the UTIs were HAIs and all patients were immunocompromised. Three patients underwent
urinary catheterization with a Foley's catheter upon admission in the emergency department and one presented for replacement of ureterostomy tubes. All *Myroides* isolates were reported to be resistant to all the tested antibiotics. Two patients were successfully treated with tigecycline (7). In our study, all of the patients had urinary catheterization with a Foley’s catheter and none of the patients had a history of urinary calculi or urinary neoplasm or underwent endourological surgery. We suggested that using Foley’s catheters may be a risk factor for the development of UTI.

*Myroides* infections were generally encountered in immunocompromised patients but immunocompetent hosts were also reported (8, 16). In literature, *Myroides* infections were shown in patients with diabetes, bladder carcinoma, chronic renal impairment, COPD, liver disease, alcohol abuse, skin ulcer, and chronic steroid use (2). In our study, three patients had UTI and three patients had colonization. All the patients were immunocompromised and they had diabetes mellitus. Cerebrovascular disease, cardiac diseases, coronary artery disease (CAD), end-stage renal disease (ESRD), chronic obstructive pulmonary disease (COPD), Asthma, neuromuscular bladder dysfunction (NBD) were found as other comorbidities for *Myroides* infection. We suggested that the *Myroides* species can be a causative agent in urinary tract infections especially in patients with diabetes mellitus due to well growth in high concentrations of glucose (2, 17) and the patients with comorbidities were prone to *Myroides* infection and colonization. It should be kept in mind that colonization of the bacteria can be a risk factor for infections (18).

In the presented study, the species of *Myroides* spp. could be identified by VITEK 2 system and MALDI-TOF-MS. These systems identified all isolates at the genus level, but they could not differentiate *M. odoratimimus* from *M. odoratus*. By using 16S rRNA sequencing, all of the isolates were identified as *M. odoratimimus*. This result indicated that 16S rRNA sequencing
can be used as a reference method to efficiently differentiate *Myroides* species in accordance with the literature (5).

The source of hospital-acquired *Myroides* spp. infections often remains unknown but infections mostly occur after exposure to a contaminated water source or in the setting of trauma (7-10, 19). In our study, to reveal the source of the bacteria, environmental samples from two adjacent ICUs and hand-swab samples from healthcare workers were taken, but all of them were negative for *Myroides* spp. Although we were not able to find any environmental source, in this study, all of the six isolates were in the same PFGE type indicating cross-transmission between cases in two months. A recent study showed that PFGE clearly differentiated *Myroides* isolates from two different periods into two possibly related clones (9). This data indicates that PFGE is a useful molecular typing method for local, short term outbreaks for detecting the clonal relationship between *Myroides* isolates. Infection control and cleaning measures, such as ensuring hand hygiene compliance, prevention of frequent use of mechanical ventilation, umbilical catheter, central venous catheter, reduction of the duration of hospital stay of patients, and prevention of improper use of antibiotics were necessary to prevent *Myroides* related hospital outbreaks. We suggested that bacteria probably persisted in the hospital in a source like water, so hospitals should have a prospective water management program.

*Myroides* spp. are extensively antibiotic-resistant but resistance mechanisms are still unclear (20). Two chromosomally-encoded Metallo-beta-lactamases, TUS-1 for *M. odoratus* and MUS-1 for *M. odoratiminimus*, are considered to involve in resistance against beta-lactams (21). Many strains have been recognized as resistance to beta-lactams, monobactams, carbapenems and aminoglycosides (2,7). Due to their extensively drug-resistance, appropriate antibiotic treatment can be quite challenging. Quinolones combined with rifampicin is reported to be appropriate drugs for treatment. On the other hand, tigecycline, meropenem, cotrimoxazole, or piperacillin/tazobactam resulted in favorable clinical outcomes (7). In the presented study, only
patient P1 was successfully treated with piperacillin/tazobactam. However, one patient receiving meropenem-colistin treatment and another patient P5 receiving tigecycline and fosfomycin treatment died. We suggest that fast and reliable identification methods at species level and antibiotic testing methods are important for routine microbiology laboratories.

In conclusion, *M. odoratimimus* can cause urinary tract infections and lead to hospital acquired outbreaks. Diabetes mellitus and the use of Foley’s catheter were common risk factors for *M. odoratimimus* infections. Because *Myroides* species show multi-drug resistance and can cause difficult-to-treat infections, fast and reliable identification methods are necessary to choose efficient therapy. When VITEK-2 and MALDI-TOF-MS systems cannot differentiate between *M. odoratimimus* and *M. odoratus*, 16S rRNA sequencing can be a reliable approach for species identification. Although *M. odoratimimus* is rarely isolated from human specimens, clinicians should be aware of its ability to cause UTIs and outbreaks.

**Conflicts of interest**

None to declare.

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### Table 1. Clinical characteristics, treatment, and outcomes of the patient with UTIs due to *Myroides odoratimimus*

| Patient no, age/gender | Department     | Admission date | Urine sample collection date | Comorbidities                                                                 | Urinary indwelling device | Prior antibiotic treatment | Type of infection /Isolated microorganism | Concomitant infections | Treatment for Myroides UTI                  | Outcome          |
|------------------------|----------------|----------------|-----------------------------|-------------------------------------------------------------------------------|---------------------------|---------------------------|--------------------------------------------|----------------------|---------------------------------------------|------------------|
| P1                     | Surgical ICU   | March 4, 2019  | March 25, 2019              | Intracranial lesion, Diabetes mellitus                                         | Urethro-vesical catheterization | None                      | UTI                                        | None                 | Piperacillin/ Tazobactam                    | Cured            |
| P2                     | Internal medicine ICU | March 7, 2019 | March 26, 2019              | Cerebrovascular disease, diabetes mellitus, hypertension, CAD, ESRD           | Urethro-vesical catheterization | Meropenem, linezolid, fluconazole | UTI | VAP, BSI / Acinetobacter baumannii            | None             |
| P3                     | Neurology ICU  | February 16, 2019 | May 13, 2019               | Cerebrovascular disease, diabetes mellitus, hypertension, COPD, Asthma, BPH, NBD | Urethro-vesical catheterization | None                      | Bladder colonization | None                 | None                                        | Favorable        |
| P4                     | Neurology ICU  | May 2, 2019    | May 20, 2019                | Hypertension, diabetes mellitus, heart failure, cerebrovascular disease | Urethro-vesical catheterization | None                      | Bladder colonization | VAP / Acinetobacter baumannii             | None             |
| P5                     | Respiratory ICU | April 17, 2019 | May 20, 2019                | Alzheimer's disease, hypertension, CAD, diabetes mellitus                     | Urethro-vesical catheterization | Meropenem, colistin, fluconazole | UTI                                        | Moxifloxacin, ceftriaxone | None                                        | Failure          |
| P6                     | Respiratory ICU | May 18, 2019   | May 23, 2019                | Heart failure, diabetes mellitus, hypertension, CAD                          | Urethro-vesical catheterization | Moxifloxacin, ceftriaxone | Bladder colonization | Pneumonia                          | None             |

**Abbreviations:** BPH, benign prostatic hyperplasia; BSI, bloodstream infection; CAD, coronary artery disease; COPD, Chronic obstructive pulmonary disease; ESRD, end-stage renal disease; ICU, intensive care unit; NBD, neuromuscular bladder dysfunction; UTI, urinary tract infection; VAP, ventilator-associated pneumonia.
Figure Legends:

**Figure 1.** A phylogram showing identity between *M. odoratimimus* strains of the current study and those in NCBI. Neighbor-joining phylogenetic tree with Kimura two-parameter distances based on 16S rRNA gene sequences showed the phylogenetic positions of our six *M. odoratimimus* labeling with their accession numbers, one *M. odoratimimus* reference strain (G13), two other species of the genus *Myroides* (*M. profundi* and *M. xuanwuensis*) and *Flavobacterium antarcticum* as an outgroup. Bootstrap confidence limits were based on 1,000 replicates.

**Figure 2.** The PFGE pattern of the six *M. odoratimimus* isolates. All isolates were in the same clone.
| Patient no, age/gender | Department            | Admission date | Urine sample collection date | Comorbidities                                      | Urinary indwelling device | Prior antibiotic treatment | Type of infection /Isolated microorganism | Concomitant infections | Treatment for Myroides UTI | Outcome |
|------------------------|-----------------------|----------------|-----------------------------|---------------------------------------------------|---------------------------|---------------------------|--------------------------------------------|------------------------|----------------------------|----------|
| P1                     | Surgical ICU          | March 4, 2019  | March 25, 2019              | Intracranial lesion, Diabetes mellitus            | Urethro-vesical catheterization                     | UTI                       | None                                        | None                   | Piperacillin/Tazobactam Meropenem, colistin | Cured    |
| P2                     | Internal medicine ICU | March 7, 2019  | 26 March, 2019              | Cerebrovascular disease, diabetes mellitus, hypertension, CAD, ESRD | Urethro-vesical catheterization                     | UTI                       | Meropenem, linezolid, fluconazole          | None                   | None                                      | Failure  |
| P3                     | Neurology ICU         | February 16, 2019 | May 13, 2019              | Cerebrovascular disease, diabetes mellitus, hypertension, COPD, Asthma, BPH, NBD | Urethro-vesical catheterization                     | UTI                       | None                                        | Bladder colonization | None                                      | Favorable |
| P4                     | Neurology ICU         | May 2, 2019    | May 20, 2019                | Hypertension, diabetes mellitus, heart failure, cerebrovascular disease | Urethro-vesical catheterization                     | UTI                       | None                                        | Bladder colonization | None                                      | Favorable |
| P5                     | Respiratory ICU       | April 17, 2019 | May 20, 2019                | Alzheimer’s disease, hypertension, CAD, diabetes mellitus | Urethro-vesical catheterization                     | UTI                       | Meropenem, colistin, fluconazole           | None                   | Tigecycline, fosfomycin               | Failure  |
| P6                     | Respiratory ICU       | May 18, 2019   | May 23, 2019                | Heart failure, diabetes mellitus, hypertension, CAD | Urethro-vesical catheterization                     | UTI                       | Moxifloxacin, ceftriaxone                 | Bladder colonization | None                                      | Favorable |

**Abbreviations:** BPH, benign prostatic hyperplasia; BSI, bloodstream infection; CAD, coronary artery disease; COPD, Chronic obstructive pulmonary disease; ESRD, end-stage renal disease; ICU, intensive care unit; NBD, neuromuscular bladder dysfunction; UTI, urinary tract infection; VAP, ventilator-associated pneumonia.
