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Outbreak

A report on healthcare-associated *Myroides odoratimimus* outbreak. Is the urine bottle guilty?

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Abstract

Introduction: The aim of this study was to report a 9-case *Myroides odoratimimus* outbreak in the intensive care units (ICUs) of a secondary care hospital.

Methodology: The hospital laboratory recorded several consecutive detections of *Myroides* spp. in urine samples in March 2023. Consequently, an outbreak investigation was initiated. Epidemiological data of each patient was collected to identify the cause of the outbreak.

Results: All patients were followed up in the ICU and all growths were found to be in urinary catheters. None of the patients had clinical symptoms of urinary tract infection. Outbreak investigation revealed that urine bottles, which should be separate for each ICU patient, were in fact used for all patients. Environmental sampling of surfaces was not performed. No clustering was observed in terms of patients regarding follow-up doctors and staff. There was no mortality among these patients during the outbreak. All strains identified as *Myroides* spp. in the hospital laboratory were identified as *Myroides odoratimimus* with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). Pulsed-field gel electrophoresis (PFGE) revealed that there were 3 PFGE groups. The clustering rate was 88.8%. When the similarity ratio between PFGE profiles was > 85, one of the 9 strains showed a unique profile; while the remaining 8 strains were classified into 2 epidemiologically related groups.

Conclusions: *Myroides* spp. represents a new threat with a broad antimicrobial resistance profile, and the potential to cause epidemics across a wide clinical spectrum from colonization to lethal infection, particularly in ICU patients.

Key words: *Myroides*; outbreak; urine bottle; superbug.

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Introduction

Myroides spp. comprises of non-motile, Gram-negative, non-fermentative, oxidase-positive, rod-like bacteria that are resistant to many antibiotics. *Myroides* spp. was previously classified as *Flavobacterium odoratum* [1]. *Myroides* spp. are widely distributed in nature, especially in soil and water [2,3]. Although new subspecies have been identified over time with the development of molecular methods, two species—*odaratus* and *odoratimimus*—are regarded to be the most relevant for most of the reported cases [4]. *Myroides* spp was previously defined as a rare pathogen; however, the bacteria can be encountered in many clinical forms, as both community-acquired and healthcare-associated infections [4,5]. So far, many different clinical forms of *Myroides*-associated infections, such as urinary tract infections, skin and soft tissue infections, sepsis, and bacteremia have been reported [6–9]. *Myroides* spp. infections are mostly opportunistic and may be associated with high morbidity and mortality. Some authors have suggested that it may be a new superbug due to its antibiotic resistance profile [10–12]. *Myroides* spp. are resistant to a broad range of antibiotics, and the antibiotic resistance varies among strains isolated from different sources [13]. The antibiotic resistance mechanisms and virulence factors of the bacteria have not yet been clearly defined. However, it is known that *Myroides* spp lack flagella and some isolates have the ability to form biofilms [13,14].

Another aspect of *Myroides* spp. that should be of concern—in addition to the antibiotic resistance profile—is that it has the potential to cause outbreaks. To date, five *Myroides*-associated outbreaks have been reported worldwide [3,15–18]. It is noteworthy that all of the reported outbreaks were urinary tract infections and the source of the outbreak could not be identified in any of them. There was no growth in environmental

cultures taken in the reported outbreaks.

Reporting outbreaks involving less common pathogens allows for a better understanding of transmission routes, risk factors of the pathogen, and revision of infection control recommendations. The aim of this study was to report a 9-case *Myroides odoratimimus* associated outbreak in the intensive care units (ICUs) of a secondary care hospital which was thought to be due to inappropriate use of urine bottles.

Methodology

This is a report of a healthcare-associated urinary tract infection outbreak. A detailed description of the outbreak was described following the Outbreak Reports and Intervention studies of Nosocomial infection (ORION) statement recommendations.

Participants and setting

The hospital where the outbreak occurred is a 370-bed secondary care hospital located in the north of Türkiye. The ICU department of the hospital consists of 5 different ICUs with a total of 38 beds. The ICU layout and the locations of outbreak cases are shown in Figure 1.

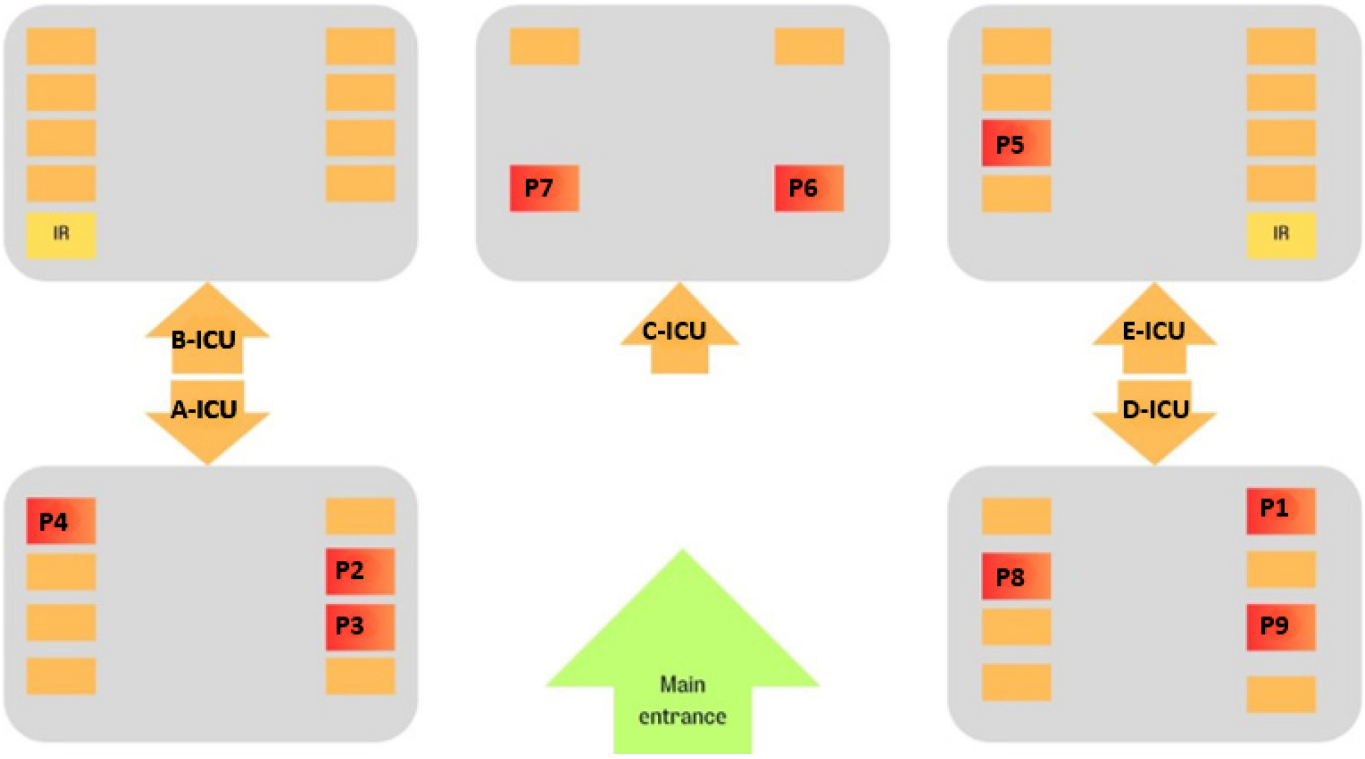
The hospital laboratory recorded several consecutive detections of *Myroides* spp. in urine samples in March 2023. *Myroides* spp. had not been detected in the hospital laboratory in the previous year. An outbreak investigation was initiated. An outbreak analysis team consisting of an infectious diseases specialist, a clinical microbiology specialist, infection control committee nurses, and intensive care nurses was set up.

The patients with *Myroides* spp. were identified. All patients were followed up in the ICUs. All of the growths were identified in indwelling urinary catheters. Epidemiological data of each patient was recorded to identify the cause of the outbreak. Demographic characteristics of the patients, comorbidities, current diagnosis, duration of hospital and ICU stay, follow-up doctors, nurses and cleaning staff, recent invasive interventions such as surgery, consultant physicians in the previous month, antibiotics taken, and prior abdominal imaging results were noted.

Blood cultures were taken from patients with *Myroides* spp. growth to detect possible bacteremia at an early stage, although they had no clinical symptoms indicative for urinary tract infection or sepsis.

During the investigation process, all ICU patients were assessed by the infectious disease specialist for the

Figure 1. Layout of the ICU and the locations of outbreak cases.



The ICU complex consists of 5 ICU sections (A, B, C, D, E) with separate entrances which are shown with orange arrows. In each ICU, the patient beds are represented as orange rectangles, and each bed is separated from the others by glass partitions forming individual rooms. Red colors show bed placements of outbreak patients and the patient numbers are written in the order in which growth was detected. P: patient; IR: isolation room; ICU: intensive care unit.

need for an indwelling urinary catheter, and urinary catheters which were considered to be unnecessary were removed after consultation with their primary physicians. Compliance to infection control measures in the ICU was checked by the infectious disease specialist and infection control committee nurses through ICU visits. Interviews about the possible causes of the outbreak were conducted with ICU staff.

Investigations revealed that urine bottles (Figure 2), which should be separate for each ICU patient, were in fact being used for all ICU patients.

Microbiological and molecular analysis

The bacterium was incubated in aerobic conditions for 18–24 hours in the hospital laboratory, and then isolated from sheep blood agar. The colonies appeared round and yellow-pigmented. The organism was initially identified as as *Myroides* spp by the BD Phoenix 100 (Becton, Dickinson and Company, Sparks, MD, USA). After the outbreak investigation was initiated, the strains were analyzed with molecular methods at the Microbiology Reference Laboratory of the General Directorate of Public Health.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS)

A MALDI-TOF-MS device (Bruker Microflex LT, Germany) with Flex Control 3.0 software was used for identifying the strains using mass spectrometry. Acetonitrile (CAN; HPLC grade; Sigma-Aldrich, St. Louis, MO, USA), trifluoroacetic acid (TFA; Sigma-Aldrich, St. Louis, MO, USA), ultra-pure water with a 0.1 µm filter without DNAse and RNAse (Sigma-Aldrich, St. Louis, MO, USA), Bruker bacterial test solution (BTS; Bruker Daltonics, Bremen, Germany) containing *Escherichia coli*, RNAase, and myoglobin protein profiles were also employed. For microbial biomass analysis using the MALDI-TOF-MS method, a single colony was taken from each isolate with the help of a sterile toothpick and placed onto a special steel 96 micro scout plate (MSP; Bruker Daltonics, Bremen, Germany; direct transfer method). This was spread onto the wells in the plate in the form of a thin film. After drying, 1 µL α-cyano-4-hydroxy cinnamic acid (CHCA; Bruker Daltonics, Bremen, Germany) matrix solution (12.5 mg/mL CHCA in a 50% ACN and 2.5% TFA mixture) was added and allowed to dry completely at room temperature. The MALDI 96 MSP was placed in the MALDI-TOF-MS device, and the system was operated using the optimized method for the identification of microorganisms in linear positive ion mode at a 2,000–20,000 Dalton (Da) mass range. A 60 Hz nitrogen laser at 337 nm was employed as the ion source. Laser pulses consisting of 40 packets of 240 were applied in the measurement of each colony in order to obtain the spectra. Each sample was studied in triplicate, and the highest readings were included in the analysis.

Figure 2. An example of the urine bottles used for urine drainage of patients in the intensive care unit.

Pulsed-field gel electrophoresis (PFGE)

All the isolates were typed by PFGE, following a previously described method with some modifications [19]. Briefly, bacterial cells were embedded in 1% low-melting-point agarose plugs (SeaKem® Gold Agarose, Lonza, Rockland, ME, USA) and lysed with lysozyme and proteinase K. Chromosomal DNA was digested with *Sma*-I (Thermo Scientific, Vilnius, Lithuania) for 2 hours according to the manufacturer's instructions. The DNA fragments were separated on 1% pulsed-field certified agarose (Lonza, Rockland, ME, USA) using a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA, USA) at 6 V/cm^2, 120° switch angle at 14 °C, first block switch time of 3.5–25.0 s for 15 hours, and the second block switch time was changed from 1 to 55 s for 5 hours. The agarose gel was stained with ethidium bromide (5 µg/mL) and visualized under ultraviolet light. DNA band profiles and cluster analysis were performed using the BioNumerics software version 7.5 (Applied Maths, Saint-Matins-Latem, Belgium) with the unweighted- pair group with mathematical average (UPGMA) method and the Dice similarity coefficient. The *Salmonella* serotype Braenderup H9812 (ATCC

BAA-664) strain was used as a molecular size indicator. The clonal relationship among isolates was evaluated using the criteria of Tenover *et al.* [20].

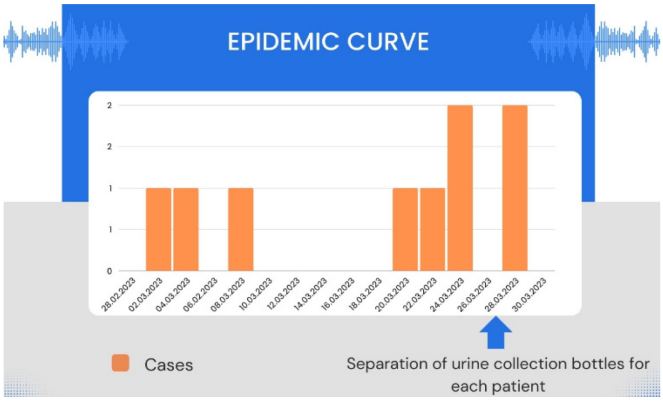
Environmental sampling

Environmental sampling of surfaces was not performed at the beginning of the investigation since it was not recommended unless supported by epidemiologic data [21]. During the course of the investigation, when we learnt about the inappropriate use of urine collection containers, and suspected it to be the source of the outbreak, the staff had already destroyed all the urine bottles; thus, we could not take samples from the suspected source of infection.

Outbreak control measures

Multiple measures were employed concomitantly for control of the outbreak. Compliance with all infection control measures in ICU, especially compliance with hand hygiene, was strictly evaluated. Unnecessary urinary catheters were removed from patients; urinary catheters of patients with *Myroides* spp. were replaced. Clean urine bottles were provided for each patient. Contact precaution was applied for all patients with *Myroides* spp. Patients transferred from the ICU to the ward were isolated in the ward. Additional training on hand hygiene, barrier precautions, and proper techniques for urinary catheter maintenance was performed.

Figure 3. Epidemic curve of healthcare-associated *Myroides odoratimimus* outbreak.



Note the decrease in outbreak cases after urine collection bottles were separated for each patient.

Results

Patients' characteristics

A total of 9 cases were identified in the outbreak, 6 males and 3 females. The epidemic curve is shown in Figure 3.

The median age was 84 years (min: 32 years, max: 94 years). All isolates were detected in samples obtained from urinary catheters. None of the patients had clinical symptoms of urinary tract infection such as

Table 1. Characteristics of patients involved in healthcare-associated *Myroides odoratimimus* outbreak.

Patient no.	Age (years)	Gender	Length of hospital stay (days)	Comorbidity	Diagnosis	Recent surgery	Urinary catheter	Prior antibiotic therapy	Urinary abnormality (USG/CT)
P1	32	Female	7	None	Intraabdominal hemorrhage	Abdominal surgery	Yes	ceftriaxone	Normal
P2	49	Male	94	None	Subdural hematoma	Cranial surgery	Yes	ceftriaxone, piperacillin tazobactam, meropenem, colistin, teicoplanin, tigecycline, TMP-SMX, vancomycin, amikacin	Normal
P3	89	Male	56	DM, HT, Alzheimer's	Pneumonia		Yes	meropenem, tigecycline, moxifloxacin, amikacin, fosfomycin	
P4	86	Female	54	HT, Alzheimer's, hypothyroidism, CHF, CVD	Acute renal disease		Yes	levofloxacin, TMP-SMX, fosfomycin	
P5	84	Male	24	COPD	Sepsis		Yes	levofloxacin, imipenem cilastatin, polymyxin b, teicoplanin	Kidney cyst
P6	83	Male	51	COPD, prostate cancer	Sepsis		Yes	imipenem cilastatin, colistin, fosfomycin	Kidney cyst, bladder cancer
P7	94	Female	16	DM, CRD	Hyperglycemia	Femur fracture surgery	Yes	meropenem, piperacillin tazobactam, ampicillin sulbactam, teicoplanin	Normal
P8	86	Male	14	COPD	ARDS		Yes	meropenem, piperacillin tazobactam, levofloxacin, vancomycin	Kidney cyst
P9	69	Male	146	None	Arrest after drowning		Yes	fosfomycin, piperacillin tazobactam, meropenem, colistin, teicoplanin, tigecycline, TMP-SMX, vancomycin, amikacin	Bladder stone

ARDS: acute respiratory distress syndrome; CHF: congestive heart failure; COPD: chronic obstructive pulmonary disease; CRD: chronic renal disease; CT: computed tomography; CVD: cerebrovascular disease; DM: diabetes mellitus; HT: hypertension; TMP-SMX: trimethoprim-sulfamethoxazole; USG: ultrasonography.

fever, hypotension etc., and all the patients were considered to be colonized with *Myroides odoratimimus*. The median length of hospital stay was 51 days (min: 7 days, max: 146 days). The patient characteristics are summarized in Table 1. No growth was detected in blood cultures of any of the patients.

Prior abdominal imaging was available for 7 of 9 patients; 4 of them had urogenital findings. All patients had a history of antibiotic therapy.

No clustering was observed in terms of patients regarding follow-up doctors, nurses and cleaning personnel, and consultant physicians in the previous month. There was no mortality among these patients during the outbreak and in the following month.

Microbiologic results

The BD Phoenix (Becton, Dickinson and Company, Sparks, MD, USA) revealed that all the strains were resistant to fluoroquinolones (ciprofloxacin and levofloxacin), beta-lactam antibiotics (ampicillin, cefazolin, ceftriaxone, ceftazidime, cefixime, piperacillin tazobactam, ertapenem, imipenem, meropenem), and amikacin. Only one strain was susceptible to trimethoprim sulfamethoxazole, and one strain was susceptible to tigecycline. All other strains were resistant trimethoprim sulfamethoxazole and tigecycline.

All strains identified as *Myroides* spp. in the hospital laboratory were found to be *Myroides odoratimimus* when identified by MALDI-TOF-MS. PFGE revealed that there were 3 different PFGE groups labelled as 1, 2 and 3 (Figure 4).

The clustering rate was 88.8%. When the similarity ratio between PFGE profiles was > 85, one of the 9 strains showed a unique profile, while the remaining 8 strains were classified into 2 epidemiologically related groups.

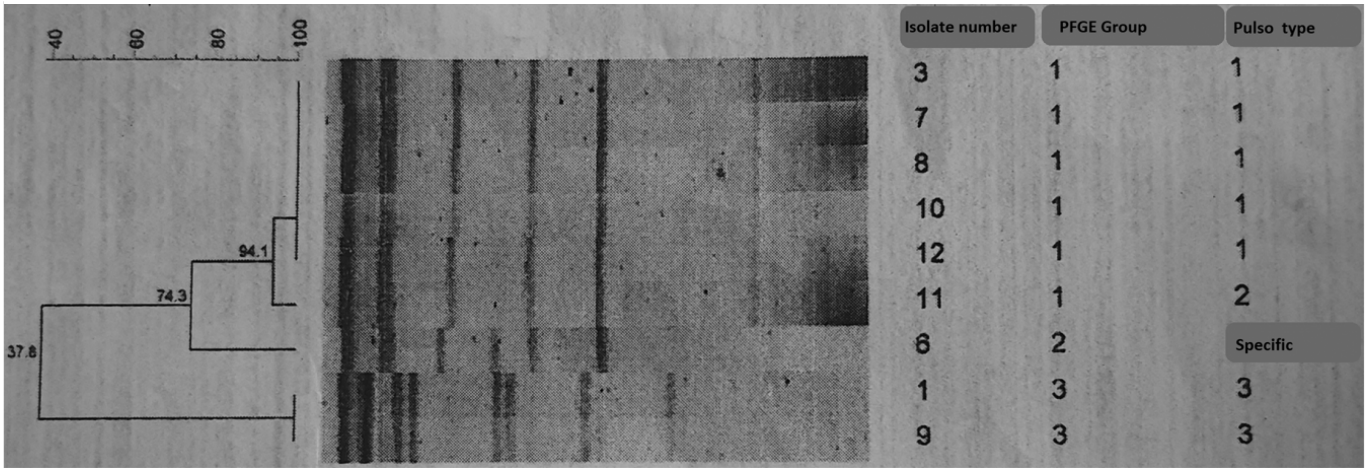
Discussion

We report a 9-case healthcare-associated urinary tract infection outbreak of multidrug-resistant *Myroides odoratimimus*. We speculate that the outbreak was caused by inappropriate shared use of urine bottles among patients, which may have led to contamination. A total of 5 *Myroides* spp. outbreaks have been reported so far; 3 of which were in Türkiye. This is the fourth report of an outbreak in Türkiye, and the sixth in the world [3,15–18]. All of the outbreaks were of urinary tract origin; some cases were symptomatic, while others involved only colonization of the bacteria, as in our outbreak. The asymptomatic course is frequently observed in *Myroides* spp. associated urinary tract infections. In a study of 228 patients evaluating *Myroides* spp. infections, 92.1% of cases were classified as asymptomatic bacteriuria [5].

When the studies evaluating *Myroides* spp. infections were analyzed, it was observed that the majority of the patients were followed up in ICU or urology wards. Aygar *et al.* and Gülmez *et al.* reported that the majority of the patients in their study were followed up in the ICU; 62% and 76%, respectively [5,12]. On the other hand, in the report by Yang *et al.*, the majority (72%) of patients were followed up in the urology service, whereas 27% of the patients were followed up in the biliary and pancreatic surgery department and had a history of abdominal surgery and drains, including those in the urology ward [22].

The source had not been identified in any of the reported outbreaks. In the case of our outbreak, we suspect that the source was the urine bottle since it was the only non-compliance we found in infection control measures. The fact that the outbreak was ceased after

Figure 4. Dendrogram of pulsed-field gel electrophoresis (PFGE) of outbreak isolates.



ensuring the appropriate use of these urine bottles is the biggest evidence in support of our speculation. Furthermore, stopping unnecessary urinary catheterizations may have helped stop the outbreak by preventing cross-contamination with healthcare personnel. Analysis of the previously reported outbreaks and case series showed that most cases involved medical devices such as urinary catheters, drains, and nephrostomy catheters [5,10–12,15–18]. Recent studies have shown that *Myroides* spp. can form relevant amounts of inherently antibiotic-resistant biofilm and colonize surfaces and tissues [23,24]. When *Myroides* spp. colonizes and forms biofilms on medical devices, patients are predisposed to recurrent and difficult to treat infections. Frequent occurrence of *Myroides* spp in ICUs and urology units, and colonization on medical devices, suggest that these bacteria may be the pathogen causing catheter-associated urinary tract infection in patients.

Although there are some common features such as immunosuppression, surgery, indwelling catheters, and diabetes mellitus among the reported cases; the risk factors for *Myroides* spp. infections have not been specifically identified [18,22,25]. However, urologic intervention and catheterization are inherent risk factors for *Myroides* spp., just like all other urinary tract infections. 91% (42/46) of patients in all reported outbreaks had a history of urinary catheterization [3,15–18]. Prior antibiotic use is a potential risk factor for *Myroides* spp., as is the case for many multidrug-resistant pathogens. However, more studies are needed to identify which antibiotics in particular are closely related to this condition. In addition, Ktari *et al.* [3] and Yagci *et al.* [16] reported a history of urinary calculi in most of their outbreak cases, whereas no such feature was observed in other outbreaks.

The fact that 4 of the 6 outbreaks due to *Myroides* spp. were reported from Türkiye is a concerning issue. Research studies involving high numbers of *Myroides* spp infection cases have been reported in Türkiye, even outside the outbreaks [5,12]. This is an indication that *Myroides* spp. is a new threat for the entire world, but more for Türkiye. This may be related to the high antibiotic use in Türkiye. The World Health Organization (WHO) reported that the median consumption of antibiotics was 38.2 DDD per 1000 inhabitants per day in Türkiye in 2018 [26]. Klein *et al.* reported the largest increase in Watch antibiotic consumption occurred in Türkiye between 2000–2015 [27]. The widespread occurrence of *Myroides* spp. infections in our country with its broad antimicrobial resistance is an important issue that needs to be investigated epidemiologically before *Myroides* spp. becomes a superbug for the entire world.

Our report has some limitations. The main limitation was that we could not collect samples from the used urine bottles since they were destroyed. We did not have the opportunity to initiate *Myroides* spp. surveillance because there were not enough staff and laboratory personnel. However, we believe that this report describes an important outbreak that was brought under control within a short time and identified the possible source.

Conclusions

We have reported a *Myroides odoratimimus* outbreak, where we suspected improper usage of urine bottles to be the source of infection, and we managed to control the outbreak within a short time.

Myroides spp. represents a new and difficult-to-treat threat with a broad antimicrobial resistance profile. *Myroides* spp. has the potential to cause epidemics across a wide clinical spectrum from colonization to lethal infection, particularly in ICU patients. It can be concluded that the most effective method of preventing and controlling outbreaks caused by this bacterium is to adhere rigorously to infection control measures.

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Authors' contributions

TK was the principal researcher. EB was a member of the outbreak investigation team and involved in the initial identification of isolates, transfer of isolates for further analysis, and interpretation of findings. ZB, HŞ, and YNÇ were involved in the further analysis of the isolates and writing the methodology section.

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Conflict of interests

No conflict of interests is declared.

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