

# Molecular Epidemiology of *Myroides odoratimimus* in Nosocomial Catheter-Related Infection at a General Hospital in China

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**Purpose:** Catheter-related infection (CRI) is one of the most frequent causes of hospitalizations for immunocompromised patients. A major challenge is the increased prevalence of *Myroides odoratimimus*. The purpose of the present study was to evaluate the clinical features and molecular characteristics of *M. odoratimimus* collected from a general hospital in Shanghai, China.

**Patients and Methods:** From July 2015 to August 2016, a total of 22 isolates of *M. odoratimimus* were collected from inpatients respectively from the biliary and pancreatic surgery (6/22) and the urology department (16/22). Clonal relatedness among the isolates was assessed using pulsed-field gel electrophoresis (PFGE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Moreover, the antimicrobial susceptibility tests were carried out using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. The presence of antibiotic resistance genes was screened using the polymerase chain reaction (PCR) assay. Additionally, protein structure prediction was analyzed using PSIPRED and RaptorX.

**Results:** PFGE differentiated these isolates into six possibly related clones from two different departments obtained during a distinct period, indicating clonal dissemination in the two departments. We compared the dendrograms of *M. odoratimimus* isolates obtained by MALDI-TOF MS with those obtained by PFGE and found that the coincidence rate between them was only 68.2%. All the *M. odoratimimus* isolates were highly resistant to most available antibiotics, including carbapenems. Furthermore, chromosome-encoded  $\beta$ -lactamases MUS-1 was confirmed by PCR in 6 of 22 *Myroides odoratimimus* isolates. Herein, we also reported a novel variant of *bla*<sub>MUS-1</sub> in the remaining 16 isolates, which encodes MUS-3 protein at position 60 (Valine to Alanine), differing from the structure of MUS-1.

**Conclusion:** The opportunistic and extensively antibiotic-resistant *Myroides odoratimimus* has a small range of epidemics in these two different departments. Clinicians should be aware that *M. odoratimimus* may induce a severe nosocomial outbreak of catheter-related infections, particularly in immunocompromised patients.

**Keywords:** *Flavobacterium*, *Myroides odoratimimus*, MUS-1, nosocomial, outbreak

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## Introduction

The *Myroides* genus was first isolated in 1923, originally classified as part of the genus *Flavobacterium*.<sup>1</sup> In 1996, the new genus *Myroides* was established, in which two species named *Myroides odoratus* and *Myroides odoratimimus* were included.<sup>2</sup> In recent years, three new species (*pelagicus*, *profundi*, and *marinus*) have been isolated

from sea water.<sup>3–5</sup> *Myroides* species are strictly aerobic and nonmotile, yellow-pigmented, oxidase-positive gram-negative rods with a characteristic fruity odor. They are ubiquitous in the environment and often found in soil and water.<sup>6</sup> In the last two decades, *Myroides* species mostly behave as rare opportunistic pathogens for humans, which may cause an increasing number of infections such as soft tissue infection,<sup>7,8</sup> necrotizing fasciitis,<sup>9</sup> cellulitis,<sup>10–12</sup> ventriculitis,<sup>13</sup> and urinary tract infections (UTIs)<sup>6,14</sup> in immunocompromised hosts. However, few cases have been recorded in immunocompetent individuals.<sup>7,8</sup> The prevalence of *Myroides odoratimimus* is increasing. This may be due to inappropriate use of antibiotics<sup>15</sup> or improved detection by reliable and practicable technologies, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).<sup>16</sup> *Myroides odoratimimus* has been gradually considered as an important nosocomial pathogen, which poses a serious health threat to immunocompromised patients and has the potential to cause a pandemic.<sup>17</sup> In the past few decades, more and more catheter-related infection (CRI) cases caused by *Myroides odoratimimus* have been reported, including three main nosocomial outbreaks of UTI caused by *M. odoratimimus*. The first main outbreak occurred in the urology ward of a Turkish hospital, which lasted for three years, from 1994 to 1997.<sup>18</sup> The second outbreak was in a Tunisian hospital from May to November 2010, also in the urology department.<sup>15</sup> The third occurred in western Romania from June to August 2017.<sup>19</sup> Here, we report on a nosocomial prevalence caused by *M. odoratimimus* in the Renji Hospital at the Shanghai Jiaotong University School of Medicine in China.

## Patients and Methods

### Bacterial Isolates and Data Collection

A total of 22 non-repetitive clinical isolates of *Myroides odoratimimus* were routinely obtained in clinical microbiology laboratory, Renji Hospital at the Shanghai Jiaotong University School of Medicine, China from July 2015 to August 2016. Of 22 isolates, 91.0% (20/22) were isolated from celiac drainage fluid, followed by urine (4.5%, 1/22) and bile (4.5%, 1/22), of inpatients undergoing aseptic abdominal surgery, admitted to two wards: the biliary and pancreatic surgery ward and the urology ward. According to the medical records, all subjects whose body fluid cultures were positive for *Myroides odoratimimus* were retrospectively reviewed based on the electronic patient hospital database and clinical microbiology

laboratory databases. The patients without obvious infection symptoms were considered as *M. odoratimimus* colonization. Data, including demographic characteristics, underlying disease, diagnosis, type of surgery, ward floor, antimicrobial use, and outcome were collected.

### Environmental Surveillance

During the same period, environmental surveillance samples were collected from various locations in the wards using a sterile swab (Copan, Italy), including the surface of the drainage tube, inner surfaces of sinks, commodes, bed rails, bedside tables, faucets, door handles, tap water, and patient's or medical staff's hands. Air sampling was performed with nutrient agar medium (Oxoid, UK) to determine the presence of *Myroides* spp.

### Identification and Antimicrobial Susceptibility

All the 22 isolates were analyzed using the VITEK2 compact (BioMérieux, France) and MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Antimicrobial resistance profiles, including piperacillin–tazobactam (TZP), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), aztreonam (ATM), ciprofloxacin (CIP), gentamicin (CN), and amikacin (AK) were determined by VITEK2 using gram-negative susceptibility cards (VITEK2 Compact AST GN13) according to Clinical and Laboratory Standards Institute (CLSI) guidelines as previously described.<sup>20</sup> Then the minimum inhibitory concentrations (MICs) of imipenem (IPM), meropenem (MEM), trimethoprim–sulfamethoxazole (SXT), and levofloxacin (LEV) were determined using the commercial broth microdilution MIC method. The MIC results were interpreted following the Clinical and Laboratory Standards Institute standards (CLSI, 2018), according to the breakpoint for non-*Enterobacteriaceae*. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* 27853 were used as quality control strains for the antibiotic susceptibility tests. The commercial broth microdilution MIC method is generally used to measure (semi-quantitatively) the in vitro activity of an antimicrobial agent against a bacterial isolate.<sup>21</sup> This assay was repeated three times, and the results were reproducible.

### Pulsed-Field Gel Electrophoresis (PFGE)

We performed molecular typing to characterize the clonal relatedness of the strains by using PFGE as previously described.<sup>22</sup> Genomic DNA was extracted from bacterial

cultures using EZ-10 spin column DNA isolation kits (Sangon, Shanghai, China) according to the manufacturer's instructions. Agarose plugs were incubated with BssHII (New England Biolabs, UK). The DNA fragments were separated by electrophoresis on 1% SeaKem Gold agarose (Lonza, Rockland, ME, United States) in 0.5 × TBE buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA; pH 8.0), using the CHEF Mapper XA PFGE system (Bio-Rad, United States) at 6 V/cm and 14 °C, with alternating pulses at a 120 angle in a 5–40 s pulse time gradient for 19 h. We analyzed the generated DNA fingerprints as recommended by Tenover et al.<sup>22</sup> The results of the PFGE homology analysis were compared with clustering analyses based on a specific mass peak using principal component analysis (PCA). Analysis was performed using the MALDI-Biotyper 2.0 software.<sup>23,24</sup>

## Detection of Antibiotic Resistance Genes

We used standard PCR conditions and appropriate primers, as listed in Table 3, to detect the presence of carbapenemase-encoding genes, including *bla*<sub>KPC</sub>, *bla*<sub>OXA</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>MUS</sub>, *bla*<sub>TUS-1</sub>, *bla*<sub>NDM</sub>, and *bla*<sub>B</sub>.<sup>25–27</sup> The resulting DNA sequences and their corresponding amino acid sequences were analyzed using the DNAMAN 6.0 program and blasted with genetic databases available on the website (<http://www.ncbi.nlm.nih.gov/BLAST/>).

## Protein Secondary Structure Prediction by PSIPRED and Tertiary Structure Modeling Analysis by RaptorX

To predict whether a point mutation could have an effect at the protein level, the Mus amino acid substitution was identified with PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred/>).<sup>28</sup>

The Mus protein modeling was analyzed with RaptorX (<http://raptorx.uchicago.edu/>) to position the mutation on the tertiary structure.<sup>29</sup>

## Statistical Analysis

Data were analyzed using SPSS TM software (IBM corp., USA) version 23.0. Chi-squared tests were used to estimate differences between groups. Statistical significance was regarded as *P* values < 0.05.

## Results

### Bacterial Isolates

We isolated 22 consecutive non-repetitive *Myroides odoratimimus* strains from immunocompromised patients with

severe underlying diseases who had undergone abdominal surgery, such as a pancreatectomy or kidney transplantation (Table 1). As preventive intraperitoneal drainage was commonly performed in these patients, 20 out of the 22 strains were isolated from abdominal drainage fluid, and only two strains were isolated from bile and urine. Of the 22 patients, 16 (72.7%) were treated at the urology department on the 16th floor, and the remaining 6 (27.3%) were treated at the biliary and pancreatic surgery department on the 13th floor. The majority of patients (68.2%) were male, and the mean age of the included patients was 48.9 years, of which 17 (77.3%) were 28–60 years old. No *Myroides odoratimimus* strains were isolated from any of the environmental surveillance samples, indicating that the above clinical isolates were likely not derived from the environment.

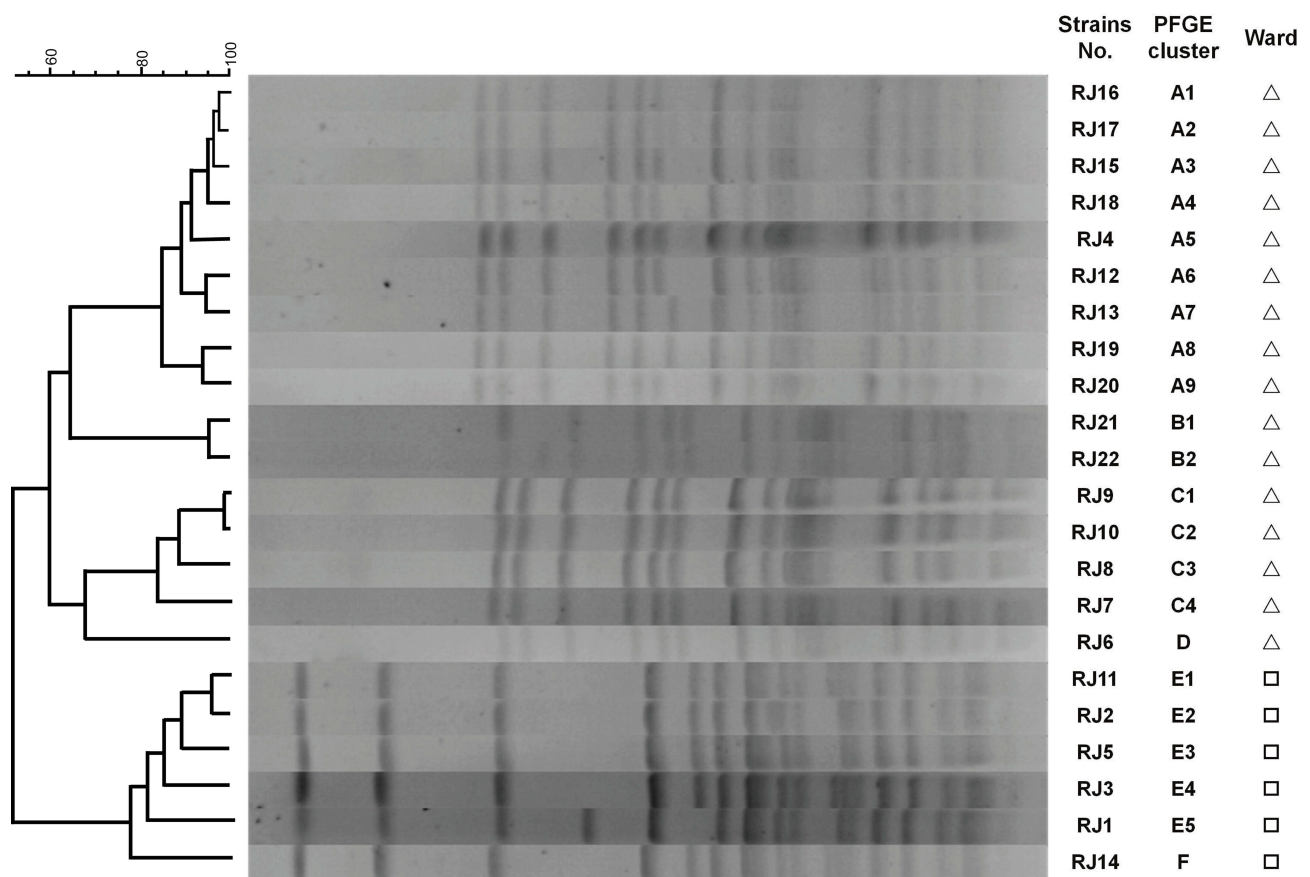
The bacterium was isolated from Columbia blood agar after 16–18h of incubation in aerobic conditions. The colonies appeared round and yellow-pigmented and had a fruity odor. The organism was initially identified using conventional biochemical reactions by the VITEK2 AES as *Myroides* isolates, but this analysis could not accurately distinguish the bacteria at the species level. We used MALDI-TOF MS to further confirm the bacterial identification as *Myroides odoratimimus* (matching score > 2.2, sequence homology > 99%) using the Microflex LT System and analyzing data on the Biotyper 2.0 software (Bruker Daltonics, Germany) for all the 22 isolates.<sup>16</sup>

### Dendrogram Analysis by PFGE and MALDI-TOF MS

According to the PFGE results, the 22 isolates belonged to six distant pulsotypes (types A–F) with a cut-off of 80% similarity (Figure 1). The dominant cluster was cluster A (40.9%, n = 9/22), followed by cluster B (9.1%, n = 2/22), which were all obtained from the urology ward during the period from September 2015 to August 2016. Cluster C (18.2%, n = 4/22) and cluster D (4.5%, n = 1/22) also came from the urology ward but were obtained between July and August 2015. Cluster E (22.7%, n = 5/22) was considered to be highly clonally-related to cluster F (4.5%, n = 1/22), both of which were isolated from the biliary and pancreatic surgery ward. MALDI-TOF MS, on the other hand, generated a dendrogram with four clusters with a cut-off value of 3 using PCA cluster analysis (Figure 2). The strains with similar PFGE patterns were divided into different branches by the MALDI-TOF MS dendrogram.

Table 1 Demographic Data of Patients Included in This Study

Patient No.	Gender/ Age	Diagnosis	Sample	Collection Date	Ward (Floor)	Infection Symptoms	Antibiotic Treatment After Operation	Outcome	PFGE	MALDI-TOF MS	MUS
RJ1	F/60	Pancreas neuroendocrine tumor	Drainage	7-Sep-15	Biliary and Pancreatic Surgery (I3F)	None	Yes	Discharged with drainage tube	E	II	MUS-I
RJ2	M/76	Hepatolithiasis	Drainage	1-Sep-15	Biliary and Pancreatic Surgery (I3F)	None	Yes	Discharged with drainage tube	E	III	MUS-I
RJ3	M/43	Pancreatic duct stones, choledochus cyst	Drainage	18-Sep-15	Biliary and Pancreatic Surgery (I3F)	None	Yes	Discharged with drainage tube	E	III	MUS-I
RJ4	F/62	Uremia, Hemodialysis	Drainage	7-Sep-15	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ5	M/47	Pancreatic head carcinoma	Drainage	25-Aug-15	Biliary and Pancreatic Surgery (I3F)	None	Yes	Favourable	E	II	MUS-I
RJ6	F/62	Uremia	Drainage	1-Aug-15	Urology(I6F)	None	Yes	Favourable	D	I	MUS-3
RJ7	M/41	Uremia	Drainage	5-Aug-15	Urology(I6F)	None	Yes	Favourable	C	III	MUS-3
RJ8	M/40	Uremia	Drainage	4-Aug-15	Urology(I6F)	None	Yes	Favourable	C	I	MUS-3
RJ9	M/62	Uremia	Drainage	23-Jul-15	Urology(I6F)	None	Yes	Favourable	C	I	MUS-3
RJ10	M/51	Uremia	Drainage	17-Jul-15	Urology(I6F)	None	Yes	Favourable	C	I	MUS-3
RJ11	M/56	Common bile duct stones, liver cancer	Drainage	7-Sep-15	Biliary and Pancreatic Surgery (I3F)	Fever	Yes	Died	E	II	MUS-I
RJ12	M/41	Uremia	Drainage	12-Jan-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ13	M/57	Renal carcinoma	Drainage	24-Jan-16	Urology(I6F)	Fever	Yes	Favourable	A	I	MUS-3
RJ14	M/52	Chronic pancreatitis and pancreatic duct stone	Bile	26-Jan-15	Biliary and Pancreatic Surgery (I3F)	Fever	Yes	Favourable	F	III	MUS-I
RJ15	M/28	Uremia	Drainage	21-May-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ16	F/53	Uremia	Drainage	1-Jun-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ17	M/31	Uremia	Drainage	1-Jun-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ18	M/57	Uremia	Drainage	4-Jun-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ19	F/38	Uremia	Drainage	4-Jun-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ20	M/36	Uremia	Drainage	4-Jun-16	Urology(I6F)	None	Yes	Favourable	A	I	MUS-3
RJ21	F/36	Uremia	Urine	22-Jul-16	Urology(I6F)	Fever	Yes	Favourable	A	I	MUS-3
RJ22	F/46	Ureteral occlusion	Drainage	8-Aug-16	Urology(I6F)	None	Yes	Discharged with drainage tube	B	IV	MUS-3



**Figure 1** PFGE image of the *M. odoratimimus* strains. The 22 isolates belonged to six distant PFGE groups (types A–F) by using a cut-off of 80% similarity (△urology department, □biliary and pancreatic surgery department).

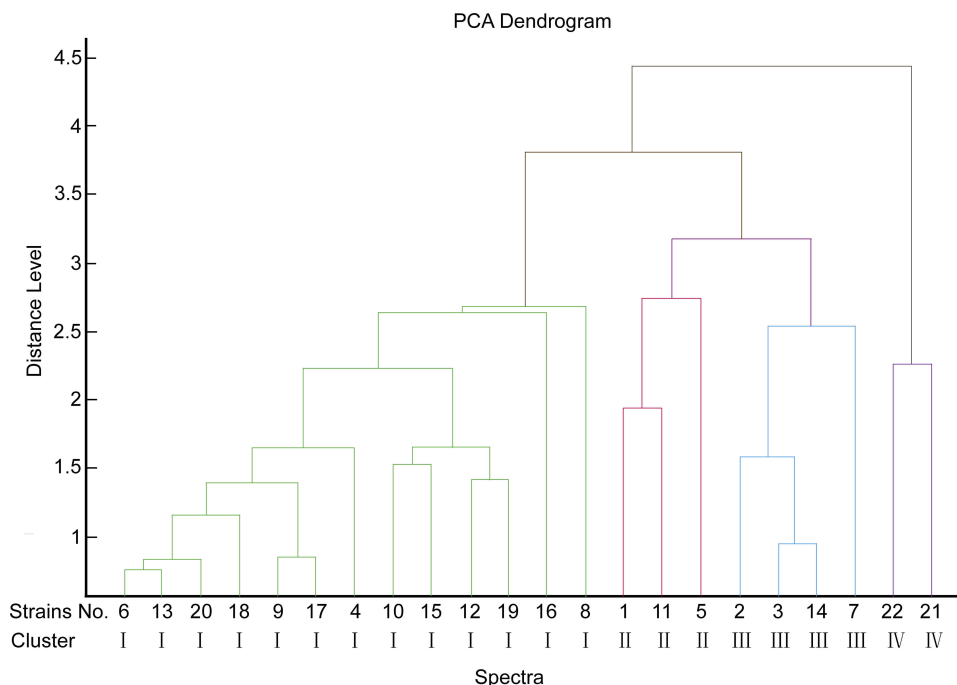
Comparing the MALDI-TOF MS data with PFGE as the gold standard, 68.2% (15/22) of strains were classified with high homology into the same cluster.

### Antibiotic Susceptibility Test

The results of antibiotic susceptibility testing revealed that all the *Myroides odoratimimus* isolates were resistant to piperacillin–tazobactam (MIC  $\geq$  128  $\mu$ g/mL), ceftazidime (MIC  $\geq$  64  $\mu$ g/mL), ceftriaxone (MIC  $\geq$  64  $\mu$ g/mL), cefepime (MIC  $\geq$  64  $\mu$ g/mL), aztreonam (MIC  $\geq$  64  $\mu$ g/mL), amikacin (MIC  $\geq$  64  $\mu$ g/mL), gentamicin (MIC  $\geq$  16  $\mu$ g/mL), and ciprofloxacin (MIC  $\geq$  4  $\mu$ g/mL). Those isolates also showed high rates of resistance to imipenem (72.7%), meropenem (54.5%), levofloxacin (77.3%), and trimethoprim/sulfamethoxazole (95.5%). The MIC results among *M. odoratimimus* isolates are shown in Table 2. Notably, the six clinical isolates from the biliary and pancreatic surgery ward possessed a significantly higher level of resistance to meropenem compared with the 16 isolates obtained at the urology ward ( $P < 0.05$ ) (Figure 3).

### Identification of Antibiotic Resistance Genes

Carbapenemase-encoding genes were further screened by PCR using carbapenemase gene primers. The nucleotide sequence analysis was negative for *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>TUS-1</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA</sub>, and *bla*<sub>B</sub> variants. However, the chromosomal *bla*<sub>MUS</sub> gene was amplified in all the 22 isolates. The six isolates from the biliary and pancreatic surgery ward carried the *bla*<sub>MUS-1</sub> gene, while a novel missense single mutation from the 178th base transition (T178C) in *bla*<sub>MUS</sub> was identified in the 16 isolates from the urology ward. The above gene mutation produces a new MUS-1 enzyme with an amino acid mutation at the 60th position (V60A), differing from the original MUS-1 enzyme. Based on the definition used to name the novel variant enzyme MUS-2,<sup>27</sup> our new enzyme variant was designated MUS-3 after the Blast Search of the Lahey database of  $\beta$ -lactamases (<http://www.laced.uni-stuttgart.de/>), and deposited in GenBank (<https://www.ncbi.nlm.nih.gov/pathogens/submit-beta-lactamase>) under the



**Figure 2** MALDI-TOF MS dendrograms for 22 isolates. Using PCA cluster analysis to construct a dendrogram, MALDI-TOF MS generated four clusters (types I, II, III, and IV) with a cut-off value of 3.

accession number MT364441. The new *blaMUS* gene was also named as *blaMUS-3*.

## In silico Structure Prediction of Point Mutation Effect

To visualize the potential effect of the V60A mutation in the MUS-3 enzyme on the MUS structure without crystallographic data, we analyzed wild-type and mutant V60A alleles with the PSIPRED secondary structure prediction tool and the RaptorX tertiary structure modeling tool for in silico structure prediction, as previously performed.<sup>30,31</sup> A short  $\beta$ -strand was predicted to form at the mutation position in the center of the amino acid chain (Figure 4). This modification is prone to result in differential enzyme activity in the MUS V60A mutant protein. Moreover, since the observed valine-to-alanine substitution is linked to the secondary and tertiary structure of the protein, the active site of the MUS enzyme may be located in the proximity of this mutation.

## Discussions

In our present work, we analyzed sampled from 22 patients with *Myroides* spp. infection/colonization who had been hospitalized long-term due to several underlying diseases and complicated abdominal surgery. After the operation, drainage tubes were routinely used to prevent

ascites formation and to reduce the incidence of abdominal infection. Multiple antibiotics had also been used to prevent opportunistic infection. Therefore, the patients were at risk of acquiring multidrug-resistant infections.

The 22 non-repetitive *Myroides odoratimimus* strains were obtained from patients in two surgery departments between July 2015 and August 2016. The majority was obtained from the urology department (72.6%), while 27.3% of strains were obtained from the biliary and pancreatic surgery department. From the electronic medical records and communications with physicians, we deduced that four patients were diagnosed with *M. odoratimimus* infections with symptoms such as fever, local swelling, and pain. These patients were treated with imipenem and levofloxacin, but one of them had a poor prognosis. The remaining patients who were identified as *M. odoratimimus* colonization did not receive any drug treatment. After extubating, patients either left or stayed in outpatient service. However, weakened hosts have an increased likelihood of developing an infection following colonization. Spanik et al reported four cases of catheter-related bacteremia caused by *Myroides* for whom the infection symptoms disappeared after removal of the catheter.<sup>32</sup> These findings are very similar to our case, and we speculate its virulence seems to be relatively low. Susceptibility data revealed *M. odoratimimus* was highly resistant to almost all antibiotics, including aminoglycosides

**Table 2** Summary of MIC Results Among the 22 *M. odoratimimus* Isolates

Isolate No.	MIC(mg/mL)											
	TZP	CAZ	CRO	FEP	ATM	CIP	AK	CN	IPM	MEM	LEV	SXT
RJ1	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	4	4
RJ2	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	4	4
RJ3	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	4	4
RJ4	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	8	>256	8
RJ5	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	4	8
RJ6	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	8	>256	>256
RJ7	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	4	8
RJ8	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	16	4	>256	32
RJ9	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	4	>256	16
RJ10	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	4	>256	16
RJ11	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	64	8	2
RJ12	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	>256	16
RJ13	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	>256	8	256
RJ14	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	>256	128	>256	4
RJ15	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	32	8	16	256
RJ16	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	32	8	>256	128
RJ17	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	32	8	>256	128
RJ18	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	64	8	>256	256
RJ19	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	32	8	>256	128
RJ20	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	8	>256	256
RJ21	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	16	16	>256	128
RJ22	≥128	≥64	≥64	≥64	≥64	≥4	≥64	≥16	8	4	>256	64

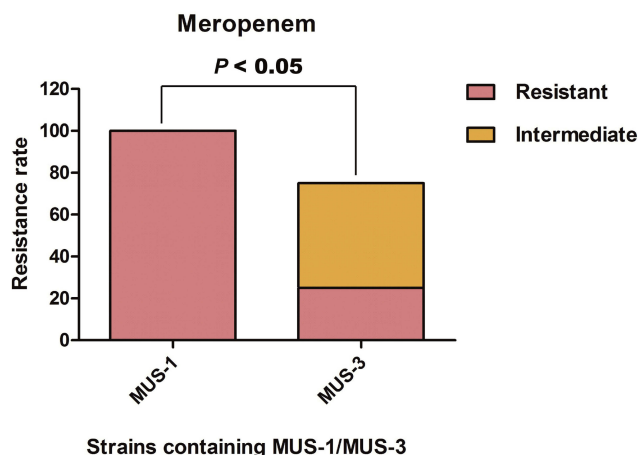
**Notes:** MIC breakpoints, imipenem (S≤4, I=8, R≥16); meropenem (S≤4, I=8, R≥16); levofloxacin (S≤2, I=4, R≥8); trimethoprim-sulfamethoxazole (S ≤2, R≥4).  
**Abbreviations:** MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, sensitive; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; CIP, ciprofloxacin; AK, amikacin; CN, gentamicin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; LEV, levofloxacin.

**Table 3** Primers for the Carbapenemase Gene Sequencing Analysis

Gene Name	Primer Name	Sequence
<i>bla<sub>KPC</sub></i>	<i>blaKPC-F</i>	5'-AGGACTTTGGCGGCTCCAT-3'
	<i>blaKPC-R</i>	5'-TCCCTCGAGCGCGAGTCTA-3'
<i>bla<sub>OXA</sub></i>	<i>blaOXA-F</i>	5'-GCGTGGTTAAGGATGAACAC-3'
	<i>blaOXA-R</i>	5'-CATCAAGTTCAACCCAACCG-3'
<i>bla<sub>VIM</sub></i>	<i>blaVIM-F</i>	5'-GATGGTGTTTGGTCGCATA-3'
	<i>blaVIM-R</i>	5'-CGAATGCGCAGCACCAG-3'
<i>bla<sub>IMP</sub></i>	<i>blaIMP-F</i>	5'-GGAATAGAGTGGCTTAAYTCTC-3'
	<i>blaIMP-R</i>	5'-GGTTTAAAYAAAACAACCC-3'
<i>bla<sub>MUS</sub></i>	<i>blaMUS-F</i>	5'-CGTTAGGCACACCAGAAGAA-3'
	<i>blaMUS-R</i>	5'-ATGCGAGTCTTACCACACCT-3'
<i>bla<sub>TUS-1</sub></i>	<i>blaTUS-1-F</i>	5'-TTAATCGTTTTAGTTGA-3'
	<i>blaTUS-1-R</i>	5'-TTATAATTGCTTGTGTT-3'
<i>bla<sub>NDM</sub></i>	<i>blaNDM-F</i>	5'-ATGGAATTGCCAATATTATGC -3'
	<i>blaNDM-R</i>	5'-TCAGCGCAGCTTGTCCGC -3'
<i>bla<sub>B</sub></i>	B-F	5'-TAAATATGCCGCTAATGC-3'
	B-R	5'-CCCCAGGTCTTTGAATC-3'

and cephalosporins, and showed decreased susceptibility to carbapenems, sulfamethoxazole–trimethoprim, and fluoroquinolones. In our research, we found that 72.7% of *M. odoratimimus* isolates were resistant to imipenem and 54.5% to meropenem. This suggests that meropenem may be more effective than imipenem for the treatment of *M. odoratimimus* infections, which is consistent with previous research.<sup>9,33</sup>

Starting from these preliminary clinical and experimental findings, in the second phase of this study, we molecularly typed all the 22 strains by PFGE and MALDI-TOF

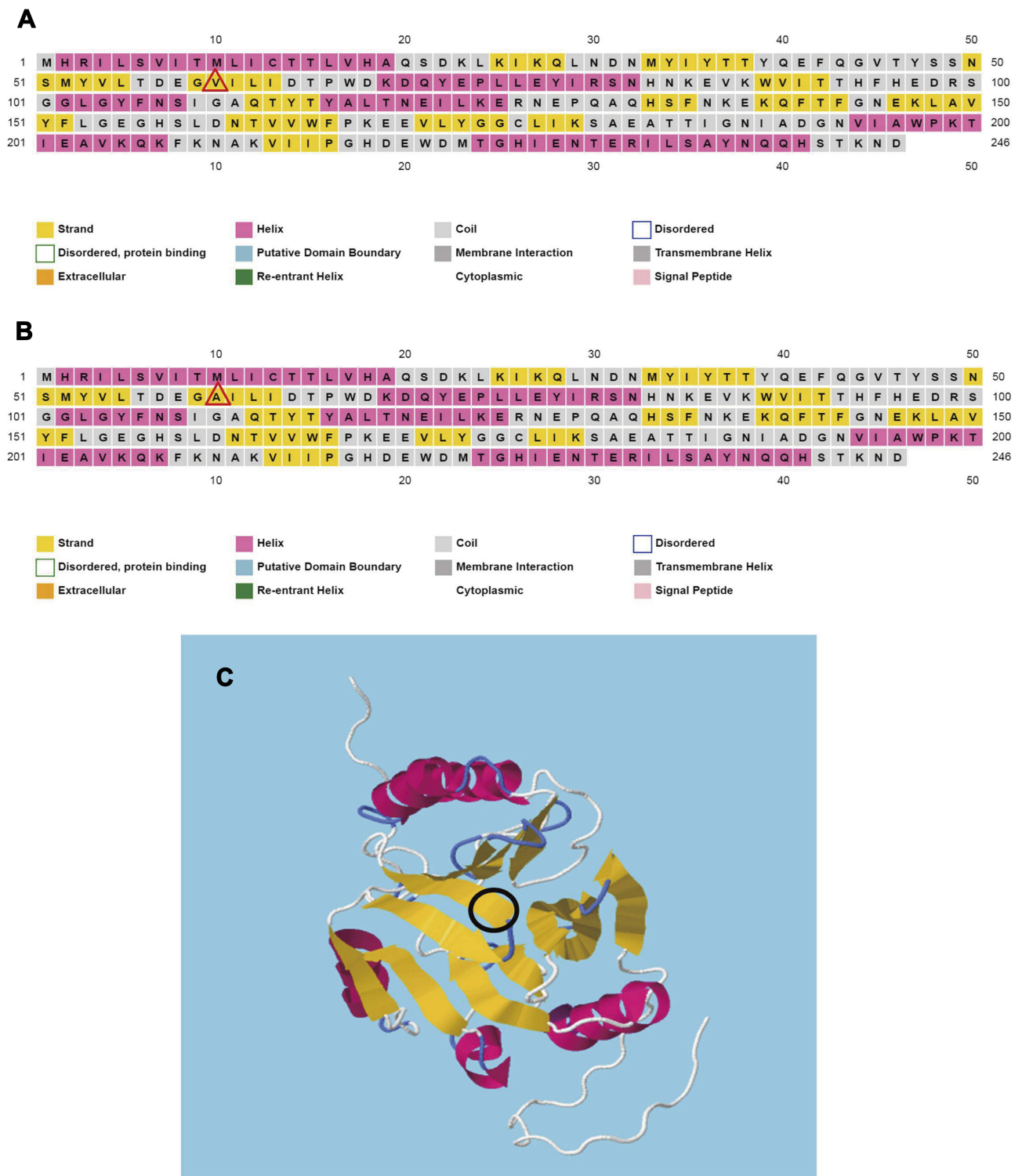


**Figure 3** The level of resistance to meropenem between the clinical isolates of MUS-1 from the biliary and pancreatic surgery ward and MUS-3 from the urology ward ( $P < 0.05$ ).

MS. We identified a total of 6 PFGE types in the 22 *Myroides odoratimimus* isolates using a cut-off of 80% similarity.

This analysis revealed that four related clusters A, B, C, and D were isolated from the urology ward on the 16th floor, while clusters E and F were obtained from the biliary and pancreatic surgery ward on the 13th floor. This suggests the potential intrahospital clonal transmission within these two departments. We are considering other strategies to prove this assumption further in future work. PFGE is the current “gold standard” fingerprinting method used to identify epidemic strains in clinical microbiological laboratories.<sup>34,35</sup> Nevertheless, there are some limitations of PFGE, for instance, it is time-consuming, and DNA restriction patterns can vary slightly between technicians or between time and place.<sup>22,36</sup> Thus, this method is suitable for regional surveillance in which the isolates have to be compared in one laboratory. MLST is emerging as an alternative DNA fingerprinting technique, particularly when information regarding evolutionary history is required. MLST is not as discerning as PFGE. The sequence types (ST) need to be defined and can be exchanged between laboratories worldwide.<sup>37</sup> However, MLST is the standard method for epidemiological investigation with large-scale international comparisons.<sup>38</sup> Whole genome sequencing (WGS) could be an alternative for molecular epidemiological research of bacteria.<sup>39</sup> WGS could obtain the entire sequence of a bacterial genome in a short turnaround time at an affordable cost. This greatly increases the amount of information that can be used to compare bacterial strains and, therefore, improves the discrimination of bacterial typing. The most frequently used WGS-based bacterial typing methods are based on single-nucleotide polymorphism (SNP) detection and core genome MLST (cgMLST).<sup>40</sup> However, here, both MLST and cgMLST are not applicable for genotyping *M. odoratimimus* since this species is a rare opportunistic pathogen in humans, and there is minimal information of ST types for this species. SNP analysis, which could exploit the whole genome positions, has the advantage that it discriminates closely-related isolates and, consequently, can perform detailed epidemiological research into clonal transmission in *M. odoratimimus*.<sup>41,42</sup> At present, the most important limitation for applying WGS-based methods in hospital epidemiological monitoring is the lack of established guidelines to determine bacterial correlation, similar to the guidelines for PFGE.<sup>43</sup> On the other hand, MALDI-TOF MS provides PCA-based dendrograms, which were





**Figure 4** The secondary and tertiary structure prediction of MUS-1 wild-type and V60A-mutant proteins using bioinformatics tools. **(A)** Secondary structure prediction of the MUS wild-type protein by PSIPRED. **(B)** Secondary structure prediction of the MUS-3 (V60A) mutant protein by PSIPRED. A beta-strand is predicted to form at the mutation position (red triangle). **(C)** Tertiary structure prediction of the MUS protein by RaptorX, with the mutation marked inside (black oval).

evaluated in a few bacterial species.<sup>23,44</sup> In the present investigation, we also used MALDI-TOF MS for the fingerprinting of 22 *M. odoratimimus* strains and evaluated the results from the two used methods. The result of the homology analysis by MALDI-TOF MS was consistent with that of PFGE by 68.2% (15/22). This shows that the PCA in MALDI-TOF MS has insufficient ability to analyze the clonality of *M. odoratimimus*. Therefore, even though MALDI-TOF MS typing is simple, fast, and cost-effective,<sup>23</sup> PFGE is more appropriate to define clonal relationships. PFGE uses genetic characteristics for strain classification, while MALDI-TOF MS adopts proteomics for phenotype analysis. On top of that, the operating procedures of MALDI-TOF MS have not yet been standardized, and even culture medium types could result in low reproducibility for identification scores. Given the above issues that could influence MALDI-TOF MS dendrograms, we argue that, at present, MALDI-TOF MS should not be substituted for PFGE to define clonal relationships.

*Myroides* spp. are multidrug-resistant microorganisms, which have been reported to have a decreased susceptibility to  $\beta$ -lactams, including carbapenems, in many previous studies. However, the molecular mechanism behind this carbapenem resistance is not clear.<sup>33,45</sup> Mammeri et al first claimed that the resistance to  $\beta$ -lactams in *M. odoratus* and *M. odoratimimus* was partly due to the production of the chromosome-encoded  $\beta$ -lactamases TUS-1 and MUS-1, respectively. These are members of the B1 subclass family, according to the classification by Ambler.<sup>46,47</sup> MUS-2 is a novel variant of MUS-1, reported in *M. odoratimimus* isolated from livestock animals in 2015, which has 98.78% amino acid sequence homology with MUS-1 and harbors three substitutions of amino acids, including V60A, H133 T, and T224I compared with MUS-1.<sup>27</sup> In our study, *bla*<sub>MUS-1</sub> was detected from the clinical strains isolated from the biliary and pancreatic surgery department. We confirmed that decreased susceptibility to carbapenem resulted from the production of a chromosome-encoded metallo- $\beta$ -lactamase. It is noteworthy that we detected another novel variant of *bla*<sub>MUS-1</sub> in the 16 strains isolated from our urology department, leading to a single amino acid substitution (V60A), which is identical to one of the substitutions from *bla*<sub>MUS-2</sub>. Thus, we designated MUS-3 after the Blast Search of the Lahey database of  $\beta$ -lactamases and deposited it in GenBank. The new variant was named *bla*<sub>MUS-3</sub>. The amino acid sequence homology between MUS-3 and MUS-1 is 99.59%. On the other hand, the six clinical isolates with MUS-1, obtained from the biliary and pancreatic surgery

ward, possessed a significantly higher level of resistance to meropenem than the isolates with MUS-3 from the urology ward. Furthermore, based on the protein secondary structure prediction by PSIPRED and tertiary structure modeling analysis by RaptorX, we found that the single amino acid mutation (V60A) could affect the active center of the MUS protein. Given the individual differences in drug resistance phenotype and genotype, we deduct that there was clonal spread of *M. odoratimimus* within the two surgical departments in our hospital. Some research has confirmed that the  $\beta$ -lactamase gene, especially chromosome-encoded MUS, was responsible for the decreased susceptibility to carbapenems.<sup>46,48</sup> However, Mammeri et al cloned the *bla*<sub>MUS-2</sub> in *E. coli* and found that it displays much lower levels of resistance to  $\beta$ -lactamase.<sup>46</sup> Thus, it is apparent that MUS could only partly explain the intrinsic resistance of *M. odoratimimus* to carbapenem and this resistance mechanism should be investigated further.

In addition, it is noteworthy that there were two peak periods of *M. odoratimimus* detection. Eleven strains and eight strains were isolated from July to September 2015 and from May to August in 2016, respectively. We speculate that the current epidemic was due to contamination of the water system in the hospital in summer, and spread through poor hand hygiene, although environmental screens failed to reveal the source.<sup>49,50</sup> As *Myroides* spp. is often isolated from sewage, we consider that the surface of the drainage tubes, inner surfaces of sinks, commodes, bed rails, bedside tables, faucets, door handles, and tap water are the major locations for environmental sampling. In the current study, thoroughly environmental surveillance was carried out in the biliary and pancreatic and urology ward, including air sampling, object surface, patient's or medical staff's hands, since *Myroides odoratimimus* clone transmission only appeared in these two wards. However, expanding environmental monitoring to the operating room in future studies might be useful since it is a potential high-risk place for being exposed to bacteria.

One of the limitations of this study is the small sample size, *Myroides odoratimimus* is rarely isolated in patients, making studies with larger sample sizes difficult to perform. We did obtain reliable statistical information of PFGE and MALDI-TOF MS based on the 22 clinical samples in this study. However, given the small sample size, it is difficult to form any definitive conclusions at present. Further studies with larger sample sizes are imperative to confirm our data.

## Conclusion

In summary, over the past decade, an increasing number of infections caused by *M. odoratimimus* has been recognized. *M. odoratimimus* are environmental organisms and implicated as pathogens of nosocomial infection. These bacteria cause opportunistic infections in immunocompromised patients, especially in catheter-related individuals. Physicians should recognize that bacteria can spread in a hospital and even cause nosocomial outbreaks. Due to the organism's multiple drug resistance, appropriate treatment options are limited. To find a more effective antibiotic treatment, extended drug sensitivity tests should be carried out. The mechanisms of drug resistance in *M. odoratimimus* are complex and have not been clarified. More research is needed to confirm the mechanisms behind drug resistance.

## Abbreviations

CRI, catheter-related infection; PFGE, pulsed-field gel electrophoresis; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; CLSI, Clinical and Laboratory Standards Institute; PCR, polymerase chain reaction; UTIs, urinary tract infections; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; CIP, ciprofloxacin; CN, gentamicin; AK, amikacin; MIC, minimum inhibitory concentrations; IPM, imipenem; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; LEV, levofloxacin; PCA, principal component analysis; T, Thymine; C, Cytosine; V, Valine; A, Alanine; MLST, multilocus sequence typing; ST, sequence types; WGS, whole genome sequencing; SNP, single-nucleotide polymorphism; cgMLST, core genome MLST.

## Compliance with Ethical Standards

This study was approved by the Ethics Committee of Renji Hospital in accordance with the 1964 Helsinki Declaration and its amendments or comparable ethical standards. The number of the ethics approval is RA-2019-198. The written informed consent was waived due to the noninterventive and retrospective nature of the study, that the collection of *M. odoratimimus* isolates was a part of the routine hospital laboratory procedures, all patient data were analyzed in anonymity, this retrospective study did not directly interfere with any patient and there was no adverse effect on the rights of patients.

## Disclosure

The authors declare no conflict of interest.

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