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Detection of *Burkholderia gladioli* in contaminated ultrasonic coupling agent

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Abstract

Background In July 2023, *Burkholderia gladioli* was detected in a patient's ascites specimen. This pathogen had not been detected in the hospital for nearly 3 years. Therefore, an investigation was immediately launched.

Methods Microbial environmental hygiene samples were collected from the operating room environment, the ultrasonic probe, and ultrasonic coupling agents in the interventional ultrasound department. The quantitative method was used to culture and identify bacterial species. Average Nucleotide Identity was used to compare the similarity of all orthologous protein-coding genes between pairwise genomes.

Results The antimicrobial susceptibility spectrum of *B. gladioli* isolated from the coupling agent was consistent with that of strains isolated from the patient sample. The two isolates were the same clone and 100% identical according to ANI. The qualified rate of non-sterile coupling agent was 18.37%; sterile coupling agents were 100% qualified. With contaminated and qualified ultrasonic coupling agent, the number of bacteria exceeding the standard rate was 88.00% and 56.00%, respectively. The qualified rate of total bacterial count of the ultrasonic probe disinfected with disinfectant wipes or ultraviolet light reached 90% or 100%, respectively. The rate of carbapenem-resistant *Acinetobacter baumannii* and carbapenem-resistant *Klebsiella pneumoniae* detected on the bedside ultrasound machine was 13.04% (6/46).

Conclusion In this case, we identified contaminated ultrasonic coupling agent and irregular use of non-sterile coupling agent. To avoid coupling agent contamination, monitoring and routine cleaning of the ultrasonic examination environment are crucial. Robust environmental testing and epidemiological investigation, along with early identification of uncommon bacteria using information system monitoring, are essential to contain an outbreak.

Clinical trial number Not applicable.

Keywords *Burkholderia gladioli*, Ultrasonic coupling agent, Ultrasonic probe, Health care-associated infection, Infection control and management

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Background

Burkholderia gladioli is a gram-negative, aerobic, non-fermenting bacterium that is widely found in water, soil, and plants [1–3]. Some studies have reported *B. cepacia* complex contamination of onions and water as well as mouthwash, sterile solution, and ultrasonic coupling agent, resulting in nosocomial infection outbreaks [3–8]. Although not a member of the *B. cepacia* complex, *B. gladioli* is a very closely related bacterium [9]. According to reports, three hospitals in Croatia have experienced outbreaks of hospital infections owing to contamination of multidose vials physiological saline with *B. gladioli* [2]. With the development of minimally invasive treatment techniques, the use of ultrasound for adjuvant therapy and surgery has become very common. However, contamination of items used in minimally invasive treatment can lead to outbreaks of infection and even catastrophic public health events, with further adverse social impacts. Therefore, these outbreaks should be detected and controlled in a timely manner [3, 10].

In July 2023, the staff of the infection management department of a large general hospital used the information monitoring system to monitor the interventional ultrasound department, and a rare bacterium, *B. gladioli*, was isolated from the ascites sample of a postoperative patient. Further investigation revealed that *B. gladioli* had not been detected at the hospital during the previous 3 years. Subsequently, an epidemiological investigation was carried out to find the reason for the presence of this bacterium.

The patient was hospitalized for confirmed hepatic metastasis of pancreatic cancer, diagnosed over 1 month earlier, and was treated with ultrasound-guided intraperitoneal injection and microwave ablation of liver lesions in July. After the surgery, cefuroxime was administered as a therapeutic medication, during which time the patient had a brief fever with a peak body temperature of 38.2℃. On the second day after surgery, the patient's abdominal drainage fluid was collected and cultured for *B. gladioli*, but no clear infection was found in laboratory testing. The patient was discharged on the fifth postoperative day after treatment.

Methods

Environmental hygiene survey in the operating room

On the basis of a literature review and epidemiological analysis of cases, it was preliminarily inferred that the infection was related to the surgical procedure, focusing on the items or fluids related to the surgical procedure.In July 2023, samples were taken from the environment and objects within the operating room of the interventional ultrasound department, including air, surgical instruments and equipment, water, sterile irrigation solution, the hands of medical staff, the ultrasonic probe, and the coupling agent.

Investigation of ultrasonic probes and coupling agents

From August 2023 to December 2023, various ultrasonic probes and coupling agents used in the hospital were sampled. The sampled ultrasonic probes included low-risk probes (such as for abdominal and cardiac ultrasound) and medium- and high-risk probes (such as for vaginal, transesophageal, cardiac, and interventional ultrasound). Ultrasonic probes were sampled after routine cleaning and disinfection. Unopened and used samples were collected from different manufacturers and different batches of coupling agent.

Sampling and detection methods

Sampling and testing were carried out in accordance with the requirements set out in the Hygienic Standard for Disinfection in Hospitals (WS/T 15982 – 2012) [11]. All surfaces of the probe were sampled, with an area of approximately 20–40 cm^2. A sterile flocking swab soaked in sterile elute was swiped over the probe, five times in each direction, and then placed in a test tube containing 10 mL of neutralizing solution. The swab was used to inoculate a common nutrient agar plate, which was cultured for 48 h at 36 ± 1℃. The number of colonies was counted, and the predominant colonies were selected for bacterial identification. For coupling agent sampling, 1 g (approximately 1 mL) of the coupling agent in use as well as unopened coupling agent was inoculated onto a common nutrient agar plate, which was cultured in the same way; the predominant colonies were selected for bacterial identification.

Evaluation criteria of results

The colony count results were divided into qualified and exceeded, according to the guidelines of the Hygienic Standard for Disinfection in Hospitals (GB15982-2012) [11] and medical ultrasonic coupling agent (YY0299-2022) [12]. The qualified colony count standards included: the total number of medium- and high-risk probe colonies was less than 20 cfu/piece, and that of low-risk probes was less than 200 cfu/piece; Pathogenic microorganisms should not be detected. Sterile ultrasonic coupling agent should meet the sterility requirements. The total number of bacteria in the non-sterile ultrasonic coupling agent was less than 100 cfu/g,*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *B. cepacia*, and *Candida albicans* should not be detected. Exceeding the above standards was defined as"exceeded".

Strain identification and drug susceptibility testing

A mass spectrometer was used for strain identification, and a compact VITEK 2 system (bioMérieux,

Marcy-l'Étoile, France) was used for drug sensitivity testing. The sensitive break point was determined according to the implementation standard for antimicrobial susceptibility tests (CLSI M100-ed33; CLSI, Malvern, PA, USA).

Whole-genome sequencing (WGS) and data analysis

High-throughput sequencing

Genomic DNA was extracted using the DNeasy®UltraClean® Microbial Kit (QIAGEN GmbH, 40724 Hilden, Germany) from strains, and a sequencing library was constructed by fragmentation, terminal repair, and addition of splices. Genome sequencing was performed on a HiSeq X sequencer (Illumina, CA, USA) using paired-end library with an average insert size of 350 bp. The disembarkation data were filtered, disjointed, and evaluated for subsequent analysis.

Sequencing data analysis

Illumina reads were quality filtered and assembled de novo using SPAdes. After completing the splicing, comparison of similarity and homology between the two strains using ANI(Average Nucleotide Identity).Drug resistance genes and other genes were annotated using Proksee (https://proksee.ca).

Statistical analysis

For bivariate analysis, chi-square test was used for categorical variables.

Results

Environmental hygiene sampling results in the operating room

The results of environmental hygiene sampling in the operating room of the interventional ultrasound department showed that *B. gladioli* was not detected in the air, water, sterile rinse solution, or sterile coupling agent or on surgical instruments and equipment, medical staff's hands, or ultrasonic probes; however, *B. gladioli* was detected in the used coupling agent as well as the unopened, non-sterile coupling agent.

Results of antimicrobial susceptibility spectrum and WGS identification of *B. gladioli*

The *Burkholderia gladioli* strain isolated from the patient and those from the coupling agent were both non-multidrug-resistant, with their antimicrobial susceptibility being completely consistent. The isolates exhibited resistance to aztreonam and colistin, moderate susceptibility to third- and fourth-generation cephalosporins, and susceptibility to the remaining antibiotics tested, including three β-lactamase inhibitors, two aminoglycosides, three carbapenems, three tetracyclines, and two fluoroquinolones. The two isolates were the same clone and 100% identical according to ANI. The two isolates were the same clone and 100% identical according to ANI. Strain Y7162672 has a 8.3 Mb genome sequence with 68.1% GC content. The comparison of the protein-coding genes revealed that *B. gladioli* pan-genome contained a total repertoire of 7,157 gene clusters (Fig. 1). After communicating with the coupling agent manufacturer, it was found that the water source storage tank in the production line had not been cleaned and disinfected for many years. After cleaning and disinfecting the water line, the number of bacterial colonies found in ultrasonic coupling agent was within the qualified range.No bacteria were detected with sterile couplers, and less than 10 cfu were detected with non-sterile coupling agent.

Sampling results of ultrasonic probe and coupling agent in the whole hospital

The detection results in the whole hospital (including coupling agent that was in use as well as unopened) showed that the qualified rate of total bacteria in non-sterile coupling agent was 18.37% (brand 1: 19.35% vs. brand 2: 16.67%), and that of sterile coupling agent was 100%. Pathogenic bacteria such as *B. gladioli* and *B. cepacia* complex were detected in brand 2 non-sterile coupling agent (Table 1). When using qualified coupling agents, the exceeded rate of the ultrasonic probe was very high (56.00%,), and when using contaminated coupling agents, the exceeded rate of the ultrasonic probe was even higher (88.00%). The specific sampling results for different groups was shown in Table 2. The qualified rate of the disinfected probe was 93.51%, and the qualified rate of the medium- and high-risk ultrasonic probes using ultraviolet disinfection was100%, all of which were sterile, with no bacterial growth. The qualified rate of the probe after disinfection with disinfectant wipes was 90% (Table 3). The chi-square test was used to compare the qualified rate of the number of colonies sampled by the ultrasonic probe after use and disinfection, and the result was $\chi^2=75.952$, $P<0.001$.

Bacterial contamination of the surface of the bedside ultrasound machine in the ward

High-frequency contact sites on the bedside ultrasound machine used for inpatients were sampled, including the ultrasonic probe, panel, handle, instrument surface, and the hands of operators of the bedside ultrasound machine. Key multi-drug resistant bacteria were screened. The detection rate of multi-drug resistant bacteria on the surface of the ultrasound machine was 13.04% (6/46). Among these bacteria were four strains of carbapenem-resistant *Acinetobacter baumannii* (CRAB) and two strains of carbapenem-resistant *Klebsiella pneumoniae* (CRKP); the results are shown in Table 4.

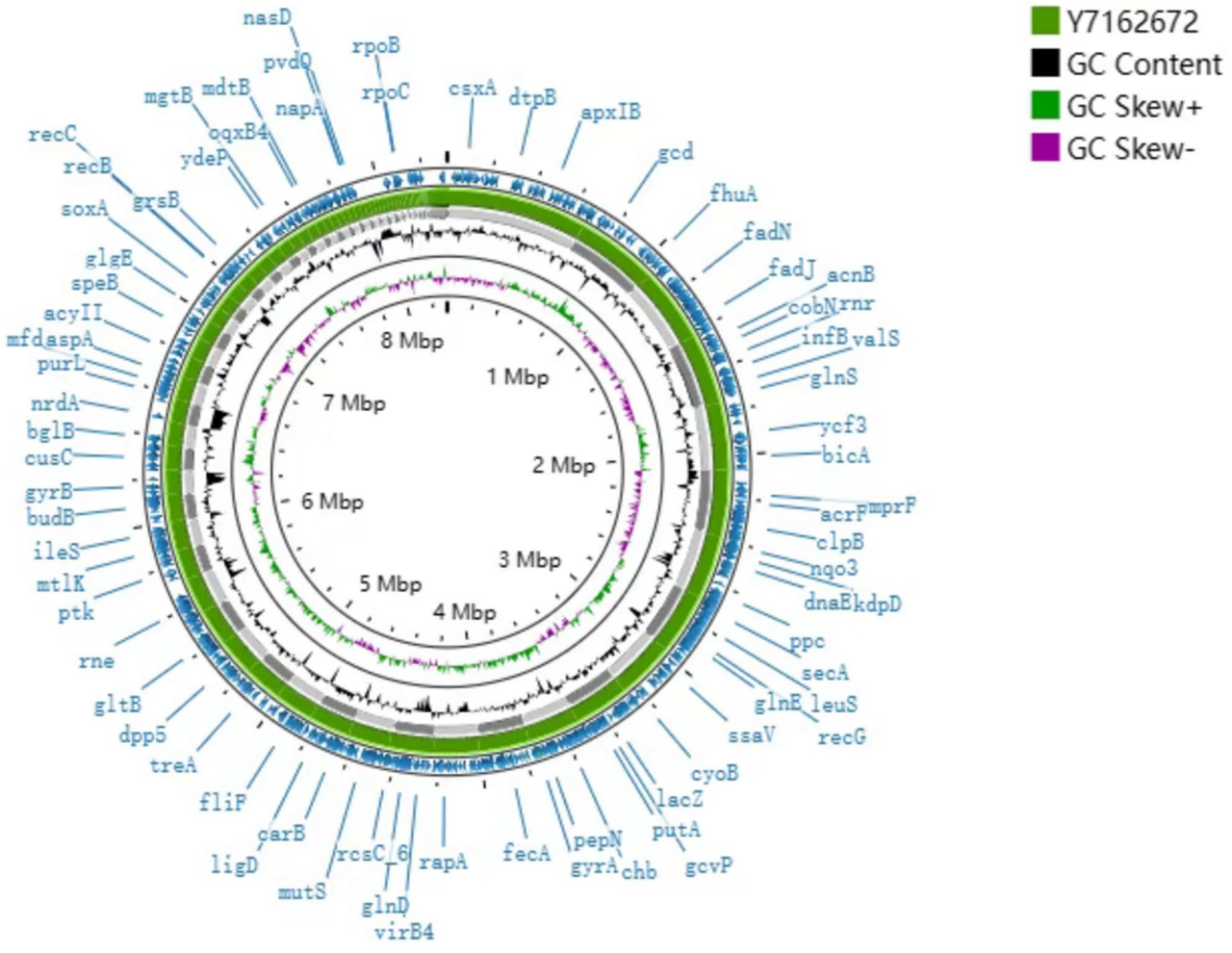

Fig. 1 Circle view of the strain Y7162672 generated by Proksee

Table 1 Sampling results of ultrasonic coupling agents

Classification of couplants	Samples	Qualified samples	Qualified rate (%)	Bacterial identification
Non-Sterile-brand 1	31	6	19.35	B. cepacia/B. contaminans
Non-Sterile-brand 2	18	3	16.67	B.gladiola/B.lata/E.coli/Delftia acidovorans/Comamonasacidovorans
Sterile	4	4	100	

Problems in field investigation and analysis of infection causes

Sterile coupling agent should be used with some medium- and high-risk ultrasonic probes, such as for esophageal ultrasound. Parts of the moderately hazardous ultrasonic probe and highly hazardous ultrasonic probe use non-sterile coupling agents when examining the patient.In this case, the non-sterile coupling agent had no qualified bacteriological test results. Some ultrasonic probes were only wiped down with paper towels after use, without disinfection.

The results of our investigation showed that the water storage tank in the coupling agent manufacturer's production line had not been cleaned and disinfected for years, resulting in contaminated coupling agent.

A non-sterile coupling agent contaminated with *B. gladioli* was used during ultrasound evaluation of the patient prior to interventional surgery. Both the probe surface and the patient's skin were contaminated with *B. gladioli*. Later, the patient's skin was not thoroughly disinfected, the ultrasonic probe was not thoroughly disinfected, or the sterile protective cover for the probe was damaged. Therefore, the operating field was contaminated *with B. gladioli*, which led to the detection of postoperative abdominal drainage in this patient.

Table 2 Different ultrasonic probe sampling results when the coupling agent used is contaminated or qualified

Ultrasonic probes classification	After use (coupling agents contamination)			After use (coupling agents qualified)			χ^2	*P*	After use (Total)		
	Samples	Exceeded samples	Exceeded Rate (%)	Samples	Exceeded samples	Exceeded Rate(%)			Samples	Exceeded samples	Exceeded Rate(%)
Medium- and high-risk ultrasonic probes	24	20	83.33	30	15	50.00	6.496	0.011	54	35	64.81
Low-risk ultrasonic probes	26	24	92.31	20	13	65.00	3.762	0.052	46	37	80.43
Total	50	44	88.00	50	28	56.00	12.698	< 0.001	100	72	72.00

Table 3 Ultrasonic probe sampling results with different disinfection methods

Ultrasonic probe classification	Disinfectant wipes disinfection			Ultraviolet disinfection			χ^2	*P*	After disinfection(total)		
	Samples	Qualified samples	Qualified rate (%)	Samples	Qualified samples	Qualified rate (%)			Samples	Qualified samples	Qualified rate (%)
Medium- and high-risk ultrasonic probe	21	17	80.95	27	27	100	3.394	0.065	48	44	91.67
Low-risk ultrasonic probe	29	28	96.55						29	28	96.55
total	50	45	90	27	27	100	1.475	0.224	77	72	93.51

Notes:"Disinfectant wipes disinfections" refers to the use of disinfectant wipes containing quaternary ammonium salt to wipe and disinfect the ultrasonic probe

Table 4 Bedside ultrasound detection of drug-resistant bacteria

Site of sampling	samples	Exceeded samples	Rate(%)	Bacterial identification
Ultrasound machines	38	5	13.16	CRAB(3), CRKP(2)
Ultrasonographers's hand	8	1	12.5	CRAB
total	46	6	13.04	CRAB(4), CRKP(2)

CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRKP, carbapenem-resistant *Klebsiella pneumoniae*

Prevention and control measures and effect evaluation

The hospital recalled and stopped using the contaminated brand of coupling agent to control the source of the infection. To standardize infection prevention and control measures in ultrasound examinations, sterile coupling should be required for all medium- and high-risk ultrasonic probes and ultrasound assessments prior to interventional surgery. The disinfection process of ultrasonic probes should be standardized, and "one disinfection after use by one person" with disinfectant wipes or ultraviolet disinfection should be strictly adhered to. The same batch of non-sterile ultrasonic coupling agent underwent further bacterial culture in the hospital, and could then be released to the clinic to use after passing all tests.

As a result of the above procedures, as of January 2024, the patient in this case has received three chemotherapy treatments and no *B. gladioli* has been detected. Additionally, no *B. gladioli* has been detected in any culture specimens from inpatients.

Discussion

B. gladioli was first reported to infect humans from respiratory secretions of patients with alveolar fibrosis [9]. Analogous to *B. cepacia* complex, this bacterium has the capacity to infect patients with chronic granulomatous disease and immunocompromised patients, making this an opportunistic pathogen. Recently, numerous studies have reported on *B. gladioli*-induced blood infections, with some studies indicating that this is among the pathogens responsible for hospital-acquired infections [9, 13–15]. In our case, the clinical medical staff failed to recognize the presence of *B. gladioli* in the patient's postoperative ascites, suggesting a lack of clear epidemiological data regarding the bacterial strain detected in the patient and insufficient knowledge concerning an abnormal infection scenario. There is a pressing need to enhance training in the prevention and control of nosocomial infection and to enhance awareness and skills among clinical medical personnel to ensure that each individual functions as a qualified practitioner of infection control. Moreover, clinical medical personnel should serve as the front-line "watchdogs" of infection control and surveillance.

The low pass rate of the non-sterile coupling agent that was unopened in this case indicates that the manufacturer lacked stringent adherence to quality control standards and failed to conduct standardized factory inspection of the product. Numerous cases of hospital infections have been reported owing to microbial contamination caused by medical ultrasonic coupling [15–19]. To mitigate the effects of media transmission resulting from

contaminated coupling agents, the national pharmaceutical equipment management agency should strictly supervise manufacturers and standardize product quality inspections. Concurrently, health care facilities should select ultrasonic coupling agents that meet health-related requirements and should ensure their safe use and management, along with periodic sampling tests, to preclude the occurrence of hospital infections.

The present case revealed that regardless of the qualification of the coupling agent used, the qualified rate of the ultrasonic probe after use remained below 50%, a finding that is consistent with the results of numerous research surveys [15, 19–21]. Currently, low- and medium-risk ultrasonic probes in medical facilities are disinfected using alcohol and cotton balls or disinfectant wipes [22]. Owing to economic and time constraints, the strict implementation of "one disinfection after use by one person" is often not feasible [23–27]. Although some medium-risk ultrasonic probes are protected by condoms or sterile sleeves, the use of unsterilized or minimally sterilized probes still poses a risk of cross-transmission of infection [28]. Given that the breakage rate of condoms ranges from 0.9 to 9%, contaminating microorganisms can potentially pass through broken condoms or incomplete closures, leading to the spread of infection [25]. During the treatment process, the ultrasonic probe comes into contact with the patient's skin, mucous membranes, body cavities, and even sterile organs and tissues. Unclean probes can contaminate these areas; secretions, body fluids, blood, and so on can also contaminate the ultrasonic probe, inevitably leading to related cross-infection issues [26]. After implementing the intervention in this study, the qualified rate of the disinfected ultrasonic probe reached 93.51%, further demonstrating that adhering to the strict implementation of the "one disinfection after use by one person" principle for ultrasonic probes can effectively reduce the risk of cross-infection.

Various pathogenic bacteria and conditional pathogenic bacteria have been detected in ultrasonic machines and coupling agents, as demonstrated in some studies [20, 21, 26, 28, 29]. In instances where methicillin-resistant *S. aureus* has been detected, the detection rate was 13.33% [30]. We collected samples from the bedside ultrasound machine used for hospitalized patients and from the hands of medical staff, identifying key multidrug resistant bacteria (CRAB and CRKP) in both cases, with a detection rate of 13%. The transmission method of multidrug-resistant bacteria is contact transmission, making it highly likely that these bacteria can be transmitted through shared equipment, such as that used during ultrasound examinations in medical facilities [22]. Infection managers often overlook this risk in the management of multidrug-resistant bacteria. Consequently, it is crucial to train medical staff in proper hand hygiene and the disinfection of ultrasonic machines [27].

There is one limitation in our study

Postoperative follow-up of individuals undergoing outpatient surgery who received interventional ultrasound puncture was not conducted in this survey, which may lead to underestimation of the potential infections caused by coupling agent contamination.

Conclusion

This research highlights the need for heightened vigilance when this rare bacterium is clinically detected, which can potentially signal nosocomial infection. Consequently, it is crucial to conduct proper risk assessment and apply scientific reasoning for the detection of this bacterium. Timely and rational investigation should be conducted to identify the source of infection and promptly halt transmission of the pathogen.

Comparison of susceptibility of Burkholderia gladioli isolated from the patient versus coupling agent

Drugs	Cases	Coupling agent
Ticaralin / Clavulanic acid	16(S)	16(S)
Piperaclin/Tazobactam	≤ 4(S)	≤ 4(S)
Ceftazidime	16(I)	16(I)
Cefoperazone/sulbactam	≤ 8(S)	≤ 8(S)
Cefepime	16(I)	16(I)
Aztreonam	≥ 64(R)	≥ 64(R)
Imipenem	≤ 0.25(S)	≤ 0.25(S)
Meropenem	1(S)	1(S)
Amikacin	≤ 2(S)	≤ 2(S)
Tobramycin	≤ 1(S)	≤ 1(S)
Ciprofoxacin	0.5(S)	0.5(S)
Levofloxacin	1(S)	1(S)
Doxycycline	1(S)	1(S)
Minocycline	≤ 1(S)	≤ 1(S)
Tigecydine	2(S)	2(S)
Colistin	≥ 16(R)	≥ 16(R)
Sulfamethoxazole/Trimethoprim	≤ 20(S)	≤ 20(S)

Notes: S stands for Sensitive, I for Intermediate, and R for Resistant

Abbreviations

WGS	whole genome sequencing
ANI	Average Nucleotide Identity
CRAB	carbapenem-resistant Acinetobacter baumannii
CRKP	carbapenem-resistant Klebsiella pneumoniae

Author contributions

XJ L, LL H and YJ J contributed equally to this work. XJ L, LL H and YJJ investigated the outbreak, collected the data and samples, interpreted the results and was a major contributor to the writing of the manuscript. BW L collected the epidemiological and clinical data. H L collected and tested the microbiological samples. Y Z, MM D and YX L designed the study, supervised the work, interpreted the data, and revised the manuscript. All the authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was conducted at the First Medical Center of the PLA General Hospital. Microbiological testing was carried out in the investigated hospitals in July 2023, which was approved by the hospital's medical ethics committee(No: S2023-055-01).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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