Antimicrobial Resistance & Infection Control

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Investigation and control of two consecutive *Serratia marcescens* outbreaks linked to reusable LigaSure devices in an operating room

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Abstract

Background *Serratia marcescens* is an opportunistic gram-negative bacterium that is commonly associated with sudden outbreaks of various healthcare-associated infections in healthcare settings. This study describes two consecutive mini-outbreaks of *S. marcescens* in an operating room following laparoscopic sleeve gastrectomy procedures.

Methods Between December 16 and 26, 2020, five patients who underwent sleeve gastrectomy at a university hospital were re-admitted with clinical symptoms. An investigation by the infection control team was conducted to identify the source. Environmental and instrument samples were collected, and antimicrobial susceptibility testing was performed. Molecular typing using pulsed-field gel electrophoresis (PFGE) was conducted to assess the genetic relatedness of the isolates. Infection control interventions were implemented during the outbreak.

Findings During this period, four of the five patients tested positive for *S. marcescens*. A total of 31 surveillance samples were collected, isolates from the handle of one LigaSure and the grasper of another were also positive for *S. marcescens*. PFGE revealed two distinct clones, indicating two separate outbreaks. Four of the six isolates from samples of patients and environment were the same, while two isolates belonging to another clone were collected from the blood samples of patients. After discontinuation of the reused LigaSure devices and implementation of strict infection control measures, no new cases were reported.

Conclusion The two consecutive *S. marcescens* outbreaks were linked to the reuse of inadequately sterilized LigaSure devices. The implementation of targeted infection control measures successfully halted further transmission.

Keywords Laparoscopic sleeve gastrectomy surgery, *Serratia marcescens*, Outbreak, PFGE

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Introduction

Serratia marcescens is a rod-shaped Gram-negative pathogen that belongs to the genus *Serratia* and the *Yersiniaceae* family [1, 2]. *S. marcescens* can cause both opportunistic and nosocomial infections, including a wide range of clinical manifestations such as surgical site infections, pneumonia and other respiratory diseases, meningitis and urinary tract infections in both adults and children, especially in immunocompromised patients [1, 3, 4]. Over the past decade, several outbreaks of *S. marcescens* have been reported worldwide. Common reservoirs in nosocomial settings include contaminated medical devices, syringes, colonized patients, healthcare workers' hands, sinks, and even soap [5, 6]. Therefore, the early identification of infected patients and other possible sources, along with the prompt implementation of infection prevention and control measures, are critical for effectively managing the spread of this organism and eliminating the source of an outbreak.

A prevalence study has shown that surgical site infections are the most common healthcare-associated infections (HAI), accounting for approximately 31% of all HAIs among inpatients [7]. Meanwhile, the global prevalence of obesity is steadily increasing [8]. As a result, some patients choose surgery to lose weight because surgical procedures have had the highest impact on effective and permanent weight loss among patients with severe obesity. Among the various surgical options, sleeve gastrectomy and gastric bypass are the most commonly performed procedures. In this study, we report two consecutive mini-outbreaks of bloodstream infections caused by *S. marcescens* following laparoscopic sleeve gastrectomy at a university hospital. We also describe the outbreak investigation process and the infection control measures that were implemented to contain the spread.

Materials and methods

Setting and outbreak recognition

The outbreak occurred in the operating room department of a 190-bed university hospital in Isfahan, Iran, which contains six separate operating rooms. Between December 16th and 26th, 2020, five patients underwent laparoscopic sleeve gastrectomy surgery. Four of these patients were re-admitted with symptoms such as fever and chills after discharge, and one patient developed signs of sepsis during her initial hospitalization. Blood and urine samples were collected from all affected patients.

On December 30, 2020, *S. marcescens* was isolated from the blood culture of the first patient, followed by a second positive blood culture from another patient the next day. As none of the patients had prior risk factors and this was the first time *S. marcescens* had been identified in patients undergoing laparoscopic sleeve gastrectomy at this hospital, both the microbiology laboratory and the specialist surgeon notified the infection control team to investigate a potential outbreak. It is worth noting that no *S. marcescens* outbreaks had been reported in this hospital since 2017.

Investigation of the environment and staff

Immediately after the specialist surgeon's report, two members of the infection control team, which included a nurse and a medical bacteriologist, held regular meetings with the officials of both the operating room and emergency department to address the situation. During the outbreak, several temporary infection control measures were implemented to prevent further transmission. The infection control team assessed compliance with key practices, including proper sterilization and disinfection protocols, hand hygiene among healthcare workers, the use of protective clothing such as gloves, and adherence to infection prevention techniques during surgery. Additionally, microbial cultures were obtained from surgical instruments and the operating room environment. It was also decided that, prior to initiating antibiotic therapy, blood and urine cultures should be obtained from all re-admitted patients presenting to the emergency department.

Microbiology

Environmental samples, as well as samples from devices, supplies, equipment, and materials, were collected from the two operating rooms where laparoscopic procedures were performed.

These included antiseptics, components of the laparoscopic surgery set (laparoscope, needle driver, trocar, bowel grasper and surgical mesh), medical equipment (oxygen tubes, oxygen masks, surgical instruments box), surgery bed, sterile glove boxes, and the hands and mobile phones of healthcare workers (HCW), in an effort to identify potential sources of infection.

S. marcescens isolates from both clinical and environmental samples were identified based on morphological and biochemical characteristics. All isolates were stored at − 80 °C for future analysis. Antimicrobial susceptibility testing of nine antibiotics (meropenem, piperacillin/tazobactam, cefepime, ceftazidime, amikacin, gentamicin, levofloxacin and ciprofloxacin and trimethoprim sulfamethoxazole) was done by a standard disk diffusion method according to the Clinical and Laboratory Standards Institute [CLSI] (2017) guidelines [9].

Molecular typing

The genetic relatedness of *S. marcescens* isolates was investigated by pulsed-field gel electrophoresis (PFGE). The genomic DNA of the isolates and reference marker *Salmonella* serotype Braenderup strain H9812 were digested by *XbaI* endonuclease, which was performed

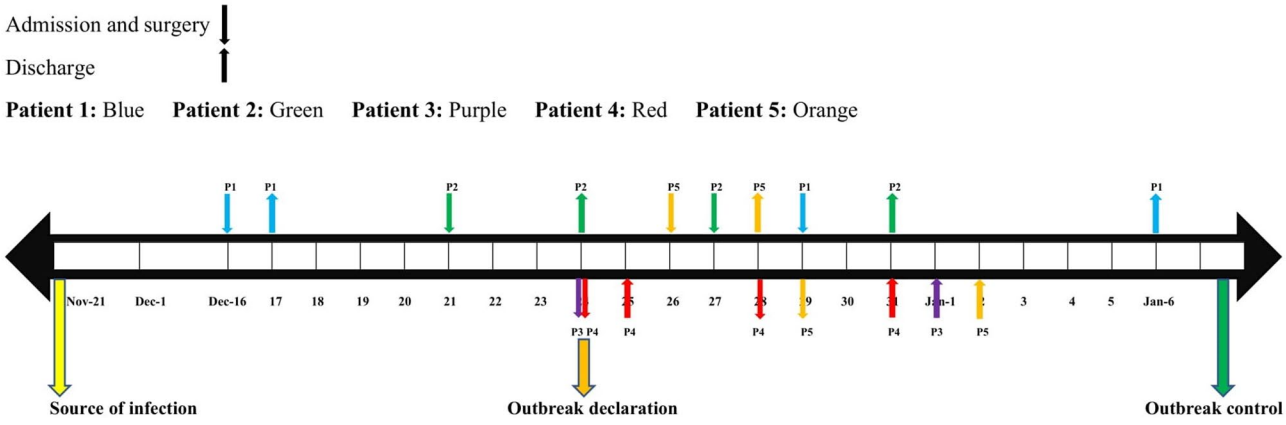

Fig. 1 Timeline of the patient's hospitalization and outbreak of *S. marcescens* in an operating room

Table 1 Clinical characteristics and outcomes of patients underwent laparoscopic sleeve gastrectomy surgery suspected of *S. marcescens* infection

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	32	36	25	25	43
Gender	Female	Female	Male	Female	Female
Body mass index, kg/m2	37.65	48.44	58.46	44.98	50.9
Type of specimen	Non	Blood	Blood	Blood	Blood
Microbial culture results	-	*S. marcescens*	*S. marcescens*	*S. marcescens*	*S. marcescens*
Surgical antibiotic prophylaxis	Cefazolin +Metronidazole	Cefazolin +Metronidazole	Cefazolin +Metronidazole	Cefazolin +Metronidazole	Cefazolin +Metronidazole
Symptoms of re-hospitalization	Fever (38 °C) and respiratory distress	Fever (38.5 °C) and chills	Fever (39.5 °C)	Fever (39 °C) and chills	Fever (38.5 °C)
Antibiotics therapy during hospitalization	Meropenem (7 days) Vancomycin (7 pays) Ceftriaxone (1 day) Clindamycin (2 days) Levofloxacin (1 day) Linezolid (1 day)	Metronidazole (3 days) Ceftizoxime (2 days)	Ceftizoxime (5 days) Clindamycin (5 days)	Metronidazole (2 days) Ceftizoxime (2 days)	Amikacin (3 days) Metronidazole (3 days) Ceftizoxime (4 days)
Systolic blood pressure	125	140	135	130	130
Diastolic blood pressure	71	90	85	70	90
O2sat at admission	83	95	95	95	84
Respiratory rate	30	18	19	20	18
Heart rate (pulse)	110	110	120	110	127
WBC	19,100	10,600	18,000	8000	5600
Neutrophil	78.8	56.6	87.2	92	89.3
Lymph	10.9	35	8.4	5.9	8.9
ESR	108	90	108	65	61
CRP	130	168	130	187	145
Length of stay in the ICU (days)	2	-	2	-	1
Length of stay in the hospital (days) (Primary/readmission)	11 days 2 / 9	9 days 4 / 5	9 days 9 / 0	6 days 2 / 4	7 days 2 / 5
Outcome	Discharge	Discharge	Discharge	Discharge	Discharge

with a CHEF-DRIII system (Bio-Rad Laboratories) as previously described (Tenover et al., 1995). PFGE patterns were interpreted according to the criteria of Tenover et al. [10].

Results

Outbreak investigation and bacterial isolation

The epidemiological, outcomes and clinical characteristics of patients involved in the two mini-outbreaks are presented in Fig. 1; Table 1. The first patient was re-admitted on December 16, 2020, after developing

clinical symptoms following laparoscopic surgery. She was empirically treated with meropenem and vancomycin; however, no microbial cultures were obtained at that time. Following this event and in response to the surgeon's report, the infection control team decided that all patients who underwent laparoscopic sleeve gastrectomy and were re-hospitalized should undergo blood culture testing prior to the initiation of antibiotic therapy. *S. marcescens* was subsequently isolated from the blood culture samples of four additional patients. The antibiotic treatment of patients is also shown in Table 1. Ceftizoxime and metronidazole was the regimen chosen to treat most patients after detection of cause of infection.

To determine the source of infection, a total of 31 surveillance cultures were obtained from operating room environments, medical instruments, supplies, and materials used in two designated laparoscopic rooms. All cultures were negative for *S. marcescens* except two: one isolate was recovered from the handle of a LigaSure device, and the other from the grasper of another LigaSure (Fig. 2). These devices had previously been used on a patient treated for an abdominal abscess and were stored inside a box containing formalin tablets for reuse in subsequent surgeries. It should be noted that all patients had undergone surgery using LigaSures that were sterilized with formalin tablets after washing.

Additionally, samples from LigaSure handle and gloves of healthcare workers revealed the presence of three microorganisms, including *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli*.

Antibiotic susceptibility testing

All *S. marcescens* isolates obtained from clinical samples and LigaSure devices exhibited identical antimicrobial susceptibility patterns. In addition, other microorganisms isolated from the handle of the LigaSure and gloves of healthcare workers included *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli*, each with distinct resistance profiles. A summary of antimicrobial susceptibility testing (AST) results for all isolates is presented in Table 2.

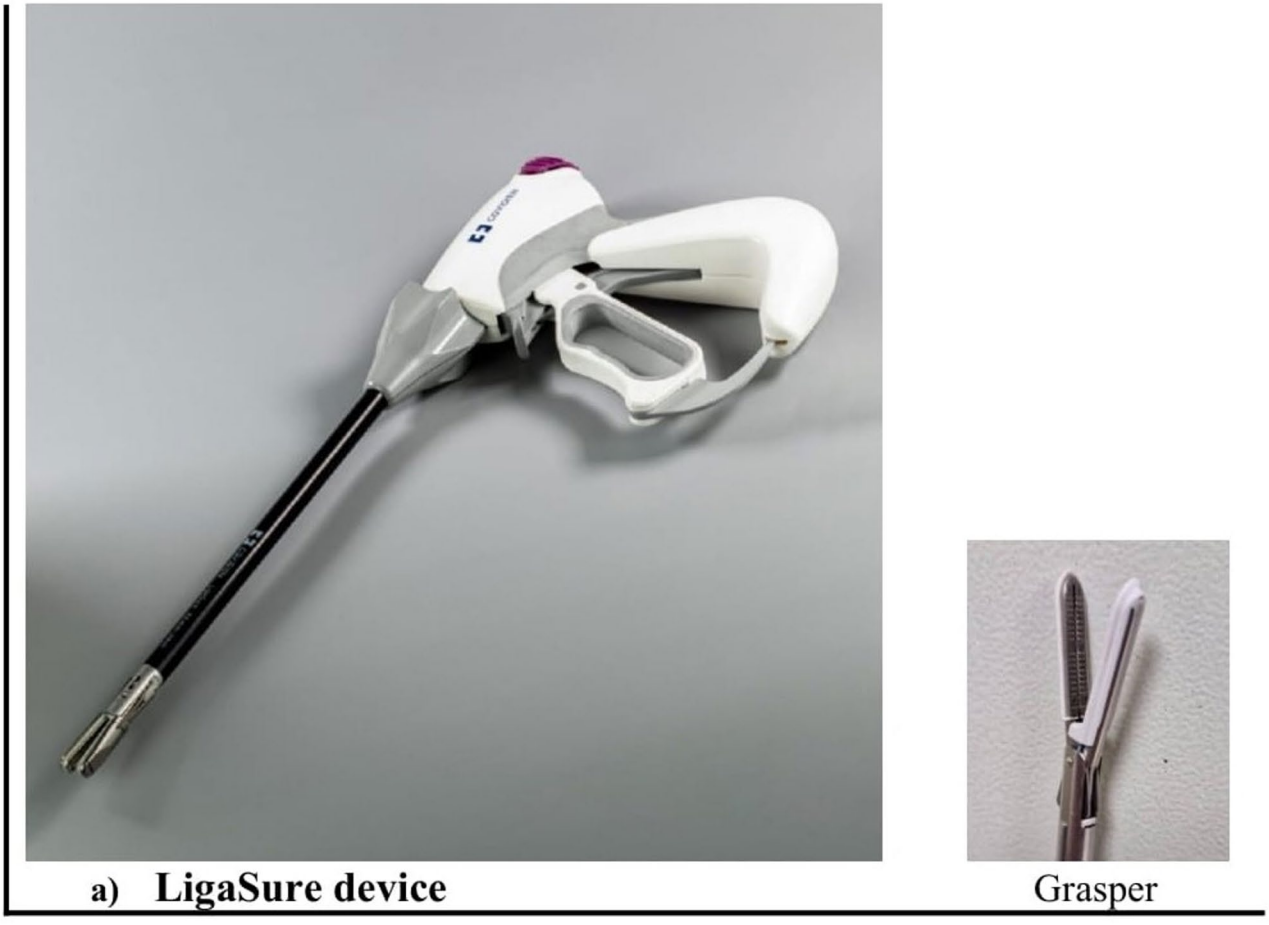

Fig. 2 The LigaSure device with highlighted sampling sites (handle and grasper)

Table 2 Antimicrobial susceptibility patterns of bacterial isolates recovered during the outbreak investigation

Organism	MEM	TZP	FEP	CAZ	AMK	GEN	LEV	CIP	SXT	COL
S. marcescens	S	S	S	S	S	S	S	S	S	NT
K. pneumoniae	R	R	R	R	R	R	NT	R	R	S
E. cloacae	R	R	R	R	S	S	NT	R	R	S
E. coli	S	R	R	R	S	S	NT	R	R	S

MEM: Meropenem, TZP: Piperacillin/tazobactam, FEP: Cefepime, CAZ: Ceftazidime, AMK: Amikacin, GEN: Gentamicin, LEV: Levofloxacin, CIP: Ciprofloxacin, SXT: Trimethoprim/sulfamethoxazole, COL: Colistin, S: Susceptible, R: Resistant, NT: Not Tested

Molecular typing

Molecular typing by PFGE was performed on six *S. marcescens* isolates, including four and two clinical and environmental isolates, respectively. Two *S. marcescens* clinical isolates (patients 3 and 5), had > 100% similarity, belonging to a unique PFGE cluster. In addition, two clinical isolates (patients 2 and 4) and two strains collected from LigaSure had 100 similarities and belonged to the same clone (Fig. 3).

Implementation of infection control measures

During the outbreak, all staff members, especially the surgeon, surgical assistants, anesthesiologists, and the operating room team, were instructed to strictly adhere to infection control measures, including surgical hand scrubbing, hand hygiene, appropriate gloves use and disinfection of reusable medical devices.

Upon detection of *S. marcescens* on the LigaSure devices, their use was immediately discontinued. The previous sterilization method using formalin tablets was replaced with plasma sterilization technology. Additionally, it was decided that LigaSure used on infectious patients should not be reused for others. Also, the infection control team emphasized that the LigaSures should be disposable as much as possible.

Following the implementation of infection control measures and microbiological investigation, these two mini-outbreaks were brought under control and no further infected patients were identified during a one-year follow-up period.

A retrospective review of laboratory records from the preceding six months revealed a case of *S. marcescens* isolated from a tissue culture sample of a patient who underwent hydatid liver cyst surgery on November 21, 2020. Notably, this isolate exhibited the same antimicrobial resistance pattern as those associated with the mini-outbreaks.

Discussion

This report describes the successfully control of two mini-outbreaks of *S. marcescens* among patients who underwent laparoscopic sleeve gastrectomy surgery in an operating room. To the best of our knowledge, this is the first LigaSures-related outbreak reported from Iran. Although our investigation could not definitively identify the source of *S. marcescens* among patients 3 and 5, we hypothesize that, in addition to contaminated surgical devices, transmission may have occurred through contact with contaminated hands or gloves of healthcare workers during surgery. This hypothesis is supported by the evidence that three cultures taken from the personnel gloves and tools were positive and multidrug-resistant (MDR) Gram-negative bacilli includes *K. pneumoniae*, *E. cloacae* and *E. coli* were isolated. In contrast to the findings of Adamson et al., who reported that MDR Gram-negative bacilli were not isolated from patients during the Serratia marcescens outbreak, our investigation identified the presence of such organisms in affected patients [11].

Several studies have shown that *S. marcescens* can persist for extended periods in the environment and contaminate medical equipment, drugs, antiseptic solutions, or water sources [11, 12, 13]. Outbreaks of *S. marcescens* from different sources have been reported in various studies around the world [6, 11]. Ersoz et al. described an outbreak of *S marcescens* meningitis after spinal anesthesia due to contaminated medications from Türkiye [14]. Three consecutive outbreaks caused by genetically unrelated clones of *S. marcescens* that were sequentially introduced from bottles of liquid theophylline and patients in the neonatal intensive care unit of the University Hospital in Switzerland reported by Fleisch et al. [15]. In South Korea, Kim et al. reported an outbreak of neurosurgical site infections associated with contaminated shaving razors [11].

According to the PFGE analysis, the *S. marcescens* population responsible for the outbreak in the operating room was categorized into two clones. The six isolates recovered included two from the LigaSure devices and four from the patients' blood samples. The presence of two distinct clones has also been reported in other studies [14, 16]. Unlike other *S. marcescens* outbreaks [13, 17], we were able to identify the source of one of the mini-outbreaks after rapid sampling of the environment, devices, supplies, equipment, and materials. Notably, there had been no reports of *S. marcescens* isolates with a similar antimicrobial resistance pattern in our hospital either two years before or one year after the outbreak.

In the present study, following the isolation of *S. marcescens* from the first patient and determination of its antimicrobial susceptibility pattern, the empiric antibiotic

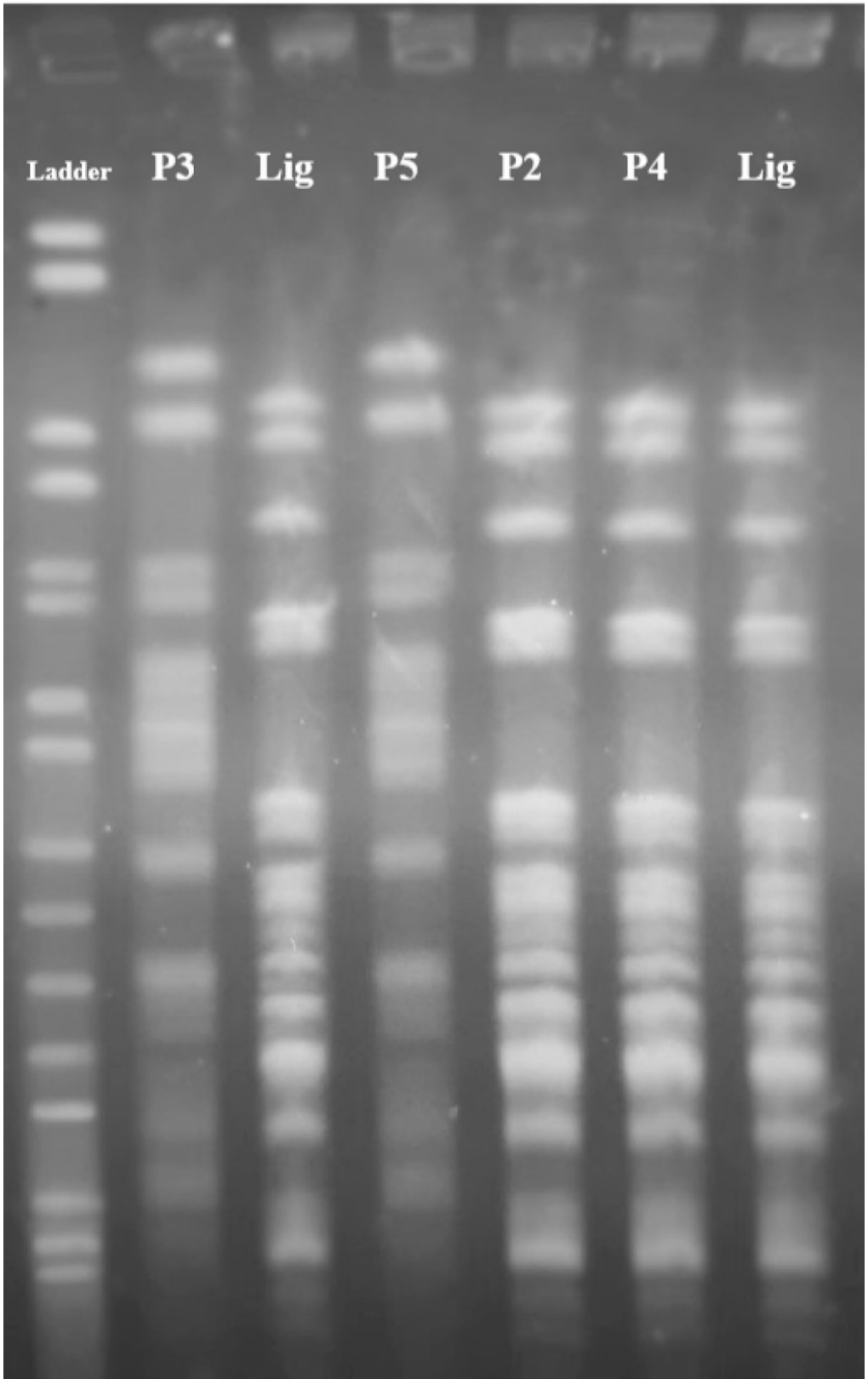

Fig. 3 Dendrogram showing results of PFGE profile of clinical isolates and environmental of *S. marcescens* in the operating room

regimen for other patients who underwent surgery and were re-hospitalized after discharge was switched from meropenem and vancomycin to ceftizoxime and metronidazole. These findings highlight that early identification of the outbreak's causative agent not only plays a key role in guiding appropriate and targeted antibiotic therapy but also contributes to reducing treatment costs and minimizing the risk of antimicrobial resistance.

It should be noted that all patients were operated by the same surgeon and when we interviewed him, we found out that he used one LigaSure for three patients in his surgeries. After the first patient's surgery, the cleaning and disinfection of the LigaSure device was observed. Due to the device's structure, it could not be fully immersed in the disinfectant solution used for precleaning. Subsequently, it was placed in a plastic box containing formalin tablets for sterilization. Therefore, it was found that instead of using a disposable ligature for one patient, the surgeon used it several times. Due to the fact that LigaSure was not cleaned well during washing, there was a possibility of contamination and too soiled, which resulted in the failure of the formalin sterilization process even if it was performed correctly.

It should be noted that all patients were operated on by the same surgeon. According to an interview with the surgeon, a single LigaSure device was used for every three patients. After the first operation, the cleaning and disinfection procedure was monitored by a nurse assistant. It was observed that due to the structural design of the device, it could not be fully immersed in the disinfectant solution. After manual cleaning, the device was placed in a plastic container containing formalin tablets for sterilization. Therefore, instead of using a disposable LigaSure for each patient, the device was reused. Since proper cleaning was not achievable due to the device's complex structure, there was a risk of residual contamination, which could compromise the effectiveness of the formalin sterilization even if it was technically performed correctly.

Although LigaSure is officially designated as a single-use device, in some low-resource healthcare settings, off-label reuse may occur due to economic and logistical constraints. This practice, while not aligned with the manufacturer's recommendations, reflects the challenges faced in under-resourced environments and represents a significant limitation of the current study.

Rapid implementation of infection control measures is essential for the early detection and containment of *S. marcescens* outbreaks. In the present study, the spread of *S. marcescens* was halted following the implementation of control measures in the operating room. Therefore, our findings highlight the importance of timely and context-specific interventions in preventing the further transmission of this pathogen in healthcare settings.

Conclusion

Our results confirmed two mini-outbreaks caused by two distinct clones of *S. marcescens* in the operating room, with four isolates from patients and two LigaSure devices. We can say that the outbreak occurred due to the lack of monitoring of the use of disposable surgical instruments by the surgeon, as well as the inappropriate implementation of instrument disinfection protocols in the operating room, which was detected and controlled by the relatively quick intervention of the infection control team. Additionally, the rapid identification of *S. marcescens* in the first patient facilitated the rational prescription of antibiotics for the other affected patients. Ongoing monitoring and enforcement of infection control programs in the operating room are crucial for controlling outbreaks of pathogenic organisms.

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Author contributions
Conceptualization, HS, SP; laboratory work, HS, VSN, MMT; collected data and analysis, MMT, VSA and MS; write-up HS; critical review, editing and formatting of manuscript HS, MMT, SP, VSN and MS supervised study: HS. All authors have read and agreed to the published version of the manuscript.

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

Declarations

Ethics approval and consent to participate
The study protocol received approval from the Ethical Committee of the Isfahan University of Medical Sciences (approval number IR.MUI.MED.REC.1403.375).

Consent for publication
Not applicable.

Permission to reproduce material from other sources
Not required.

Competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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