

Distribution and antibiotics resistance of emerging or reemerging pathogenic bacteria in 7A Military Hospital

Antibiotics resistance of emerging or reemerging pathogenic bacteria

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Abstract

Aim: The objective of this study is to determine and evaluate the distribution and antibiotic resistance of emerging or reemerging pathogenic bacteria in 7A Military Hospital (Ho Chi Minh City, Viet Nam) from January 2017 to June 2019 with the aim of contributing to clinical activities related to drug resistance, especially in emerging and re-emerging pathogens.

Materials and Methods: Bacterial strains were isolated on the MacConkey agar and identified by 20NE and 20E kit (Bio Merieux). Antibigrams were made using sensitivity discs, identification cards, and the Vitek2 Compact instrument. The procedures were performed following the guidelines of WHO and CLSI criteria for culturing and antibiogram.

Results: This study isolated 432 bacterial strains into five genera, mostly from respiratory and blood specimens. *Acinetobacter* spp. was observed mainly in the Department of Resuscitation (50.2%), had resistance against most testing antibiotics, especially aztreonam (98%), except for included colistin (18%). *Klebsiella* spp. were mostly observed in the Departments of Internal Medicine (27.3%), and Infectious Disease (27.9%) and were susceptible to many antibiotics, especially carbapenems and aminoglycoside, but were resistant against ceftriaxone (72.4%). *Stenotrophomonas* spp. mainly occurred in the Department of Internal Medicine (49.1%) and were resistant to most testing antibiotics except for SXT (10%), LEV (22%), and MIN (23%). MDR and above in *Acinetobacter* spp., *Klebsiella* spp., and *Stenotrophomonas* spp. were at 74.6%, 61.0%, and 69.8%, respectively, with XDR, reached 47.2% in *Stenotrophomonas* spp.

Discussion: *Acinetobacter* spp. and *Klebsiella* spp. were the most frequently spotted in this study and have long been known as a common pathogen. *Stenotrophomonas* spp. presence was significant, but less frequent. Their presence in blood and respiratory was expected in the medical literature. Multi-drug resistance was widely observed, especially in *Stenotrophomonas* spp., however, *Klebsiella* spp. was still highly susceptible in general. A similar range of antibiotic resistance was recorded in many other studies. Antibiotic resistance was significant especially in *Acinetobacter* spp. and *Stenotrophomonas* spp., but within literature expectation. Close monitor of emerging resistant strains and improvements in hospital hygiene is essential to prevent the risks of resistant emerging and re-emerging bacteria.

Keywords

Bacteria; Emerging; Reemerging; Antibiotics; Resistance

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Introduction

Infectious diseases have been a tough challenge for the healthcare system worldwide. Amongst the prominent issues of infectious diseases are the prolonged persistence and increased antibiotics resistance of many bacteria, which was identified by WHO as one of the three largest threats to global health [1]. It is estimated that at least 25000 deaths in Europe annually occur directly because of multidrug-resistant pathogens, and the annual economic impact is at least 1.5 billion euros [2]. Over 58000 infants die annually in India due to resistant bacteria [3]. Moreover, some microbes, which were rarely observed previously, including *Acinetobacter* spp., *Stenotrophomonas* spp., *Aeromonas* spp., and *Achromonas* spp., also have raised their prevalence recently. They are classified as emerging pathological bacteria. Other microbes such as *Klebsiella* spp., known as reemerging pathological bacteria which are well known for their pathologic effects but also had low prevalence in the past, now are reemerging at a considerable rate in clinical specimens. Emerging and re-emerging infectious diseases have recently attracted attention and awareness of both the public and the scientists, although they have long been the major threat throughout history. [2]

Initial studies have been done in Vietnam on emerging and reemerging bacteria but still in limit numbers. Therefore, we decided to perform a study aimed to determine the distribution and antibiotic resistance of emerging or reemerging pathologic bacteria in 7A Military Hospital from January 2017 to June 2019 to make a contribution to the supervision of the mentioned pathogens and provide reference data for clinical activities.

Material and Methods

Experimental objects, date, and location

This study was performed on the bacterial strains of *Acinetobacter* spp., *Klebsiella* spp., *Stenotrophomonas* spp., *Aeromonas* spp., and *Achromonas* spp. isolated from the medical specimens including blood, respiratory specimens, pus, body fluid, urine, and others at the Department of Laboratory and Pathology in 7A Military Hospital and Faculty of Biotechnology, Nguyen Tat Thanh University from January 2017 to June 2019. In total, there were 432 bacterial strains isolated during the study period.

Study methods

The bacterial strains were isolated on Mac Conkey agar. Identification was done using the 20E kit for the *Klebsiella* spp. and 20NE kit for the *Acinetobacter* spp., *Stenotrophomonas* spp., *Aeromonas* spp., and *Achromonas* spp. then verified using Vitek2 Compact (Bio Merieux) following the WHO's guidelines on conventional bacterial culturing (WHO Global Strategy for Containment of Antimicrobial Resistance 2001). The antibiogram, according to the criteria of CLSI for antibiogram (M11-A8 February 2012), was done using diffusion disks (Bio Merieux) or antibiotics cards on Vitek2 Compact.

Research ethics

The Medicine Scientific Research Ethics Committee of the 7A Military Hospital approved this study (Number: 23/QĐ-HĐYĐ-BV7A, date: 29.01.2017). The patients and relatives were well-informed about their conditions and equal treatment and were asked to take part in the study. The participation was strictly

voluntary, verified by signed documents.

This study is original and is not published in other scientific journals.

Results

Distribution of emerging and reemerging bacteria

Bacteria composition

From January 2017 to June 2019, in total, there were 432 bacterial strains isolated, belong to the *Acinetobacter* spp., *Klebsiella* spp., *Stenotrophomonas* spp., *Aeromonas* spp., and *Achromonas* spp. genus (see Table 1).

Distribution of bacteria in specimens

The *Acinetobacter* spp. strains were mostly found in respiratory specimens (118 strains, 57.6%), blood (44 strains, 21.5%) and pus (22 strains, 10.6%).

The *Klebsiella* spp. strains were mostly isolated from blood (67 strains, 43.5%) and respiratory specimens (43 strains, 27.9%).

The *Stenotrophomonas* spp. was isolated dominantly from the blood (37 strains, 69.8%), pus (6 strains, 11.3%), and respiratory specimens (5 strains, 9.4%) (Table 2).

Table 1. Bacteria composition distributed during the period of study

Bacterial strains	2017 n (%)	2018 n (%)	2019 n (%)	Total n (%)
<i>Acinetobacter</i> spp	47 (11.0)	59 (13.6)	99 (22.9)	205 (47.5)
<i>Klebsiella</i> spp	43 (10.0)	46 (10.6)	65 (15.0)	154 (35.6)
<i>Stenotrophomonas</i> spp	15 (3.5)	19 (4.4)	19 (4.4)	53 (12.3)
<i>Aeromonas</i> spp	5 (1.2)	2 (0.4)	7 (1.6)	14 (3.2)
<i>Achromonas</i> spp	2 (0.4)	0	4 (1.0)	6 (1.4)
Total	112 (25.9)	126 (29.2)	194 (44.9)	432 (100)

Table 2. Distribution of emerging and reemerging bacteria in medical specimens

Medical specimens	<i>Acinetobacter</i> spp.		<i>Klebsiella</i> spp.		<i>Stenotrophomonas</i> spp.		Others	
	n	%	n	%	n	%	n	%
Blood	44	21.5	67	43.5	37	69.8	11	55.0
Respiratory	118	57.6	43	27.9	5	9.4	3	15.0
Pus	22	10.6	16	10.4	6	11.3	2	10.0
Body fluid	11	5.4	12	7.8	2	3.8	3	15.0
Urine	9	4.4	14	9.1	3	5.7	1	5.0
Other	1	0.5	2	1.3	0	0.0	0	0.0
Total	205	100	154	100	53	100	20	100

Table 3. Distribution of emerging and reemerging bacterial strains in the Departments

Departments	<i>Acinetobacter</i> spp.		<i>Klebsiella</i> spp.		<i>Stenotrophomonas</i> spp.		Others	
	n	%	n	%	n	%	n	%
Resuscitation	103	50.2	35	22.7	13	27.3	2	10.0
Internal Medicine	38	18.5	42	27.3	26	49.1	6	30.0
Surgery	36	17.6	34	22.1	10	18.9	7	35.0
Infectious Diseases	28	13.7	43	27.9	4	7.5	5	20.0

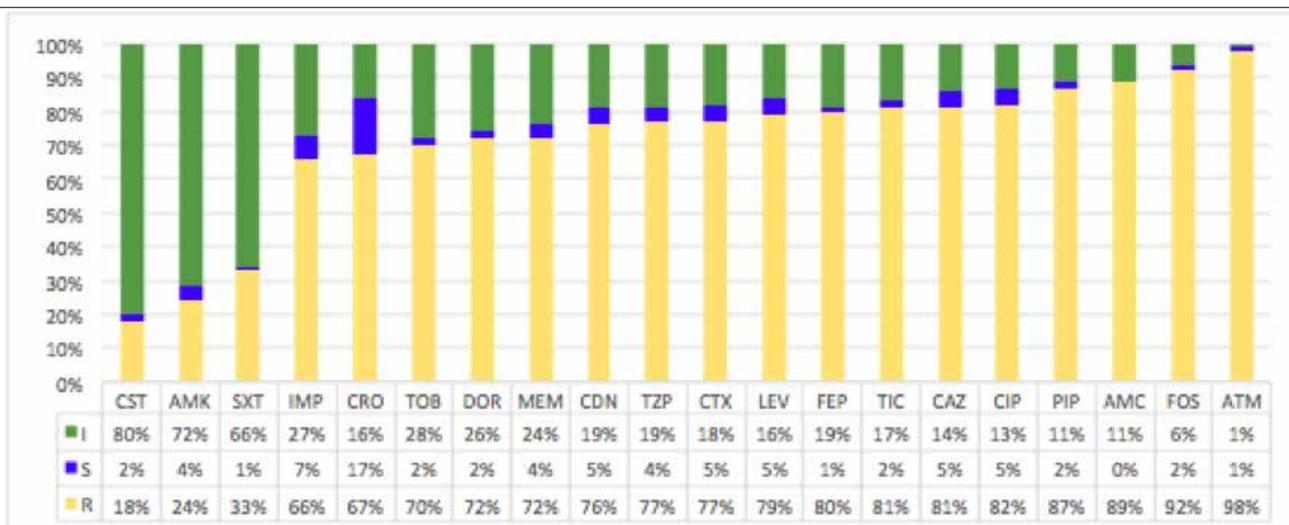


Figure 1. Antibiotics resistance of Acinetobacter spp. (S: Susceptible; I: Intermediate; R: Resistance)

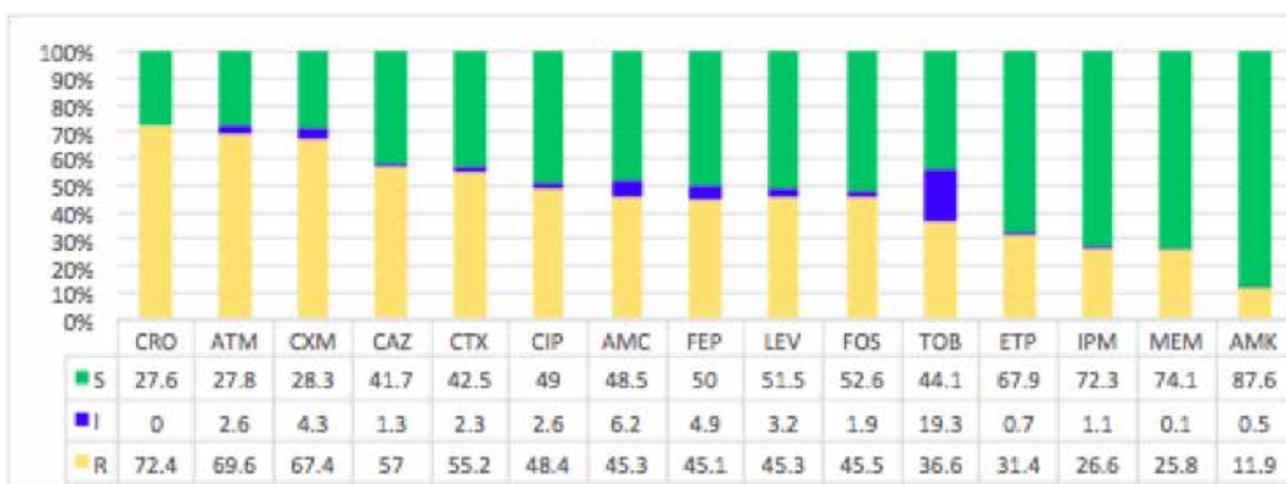


Figure 2. Antibiotics resistance of Klebsiella spp. (S: Susceptible; I: Intermediate; R: Resistance.)

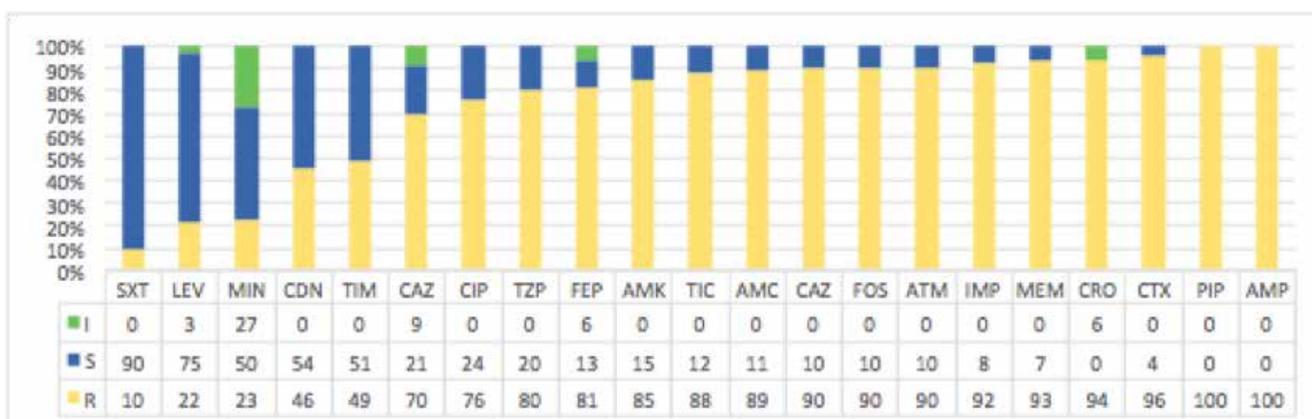


Figure 3. Antibiotics resistance of Stenotrophomonas spp. (S: Susceptible; I: Intermediate; R: Resistance.)

Distribution of bacteria per Department

The *Acinetobacter* spp. strains were mostly found in the Department of Resuscitation (103 strains, 50.2%) and the Department of Internal Medicine (38 strains, 18.5%). The *Klebsiella* spp. strains were mostly found in the Department of Infectious Diseases (43 strains, 27.9%) and the Department of Internal Medicine (42 strains, 27.3%). The *Stenotrophomonas* spp. strains were mostly found in the Department of

Internal Medicine (26 strains, 49.1%) and the Department of Resuscitation (13 strains, 27.3%) (Table 3).

Antibiotics resistance of emerging and reemerging bacteria *Acinetobacter* spp.

The *Acinetobacter* spp. strains had high antibiotics resistance, with a rate of over 50% for most of the cases. The highest resistance was against aztreonam (98%). Notable exceptions

included colistin (18%) and amikacin (24%), (see Figure 1). Ninety-eight *Acinetobacter* spp. strains (48.3%) developed multiple drug resistance (MDR, resistance against at least 3 antibiotic agents), 52 strains (25.4%) had resistance against less than three antibiotic agents, 50 strains (24.4%) had extensive drug resistance (XDR, resistance against most antibiotic agents except one or two), and 5 strains (2.4%) had pandrug resistance (PDR, resistance against all antibiotics agents).

Klebsiella spp.

The isolated *Klebsiella* spp. were susceptible to most testing antibiotics as the resistance rate was mostly less than 50%, especially to the aminoglycosides and carbapenems such as AN (susceptible rate 87.6%), MEM (74.1%), IPM (72.3%). The resistance rate over 50% was observed in CRO (resistance rate 72.4%), ATM (69.6%), CXM (67.4%), CAZ (57.0%), and CTX (55.2%), (see Figure 2).

Sixty-three *Klebsiella* spp. strains (40.9%) developed multiple drug resistance, 60 strains (39.0%) had resistance against less than three antibiotic agents, 28 strains (18.2%) had extensive drug resistance, and 3 strains (1.9%) had pandrug resistance.

Stenotrophomonas spp.

The isolated *Stenotrophomonas* spp. were highly resistant against testing antibiotics, with over 90% resistance rate against ATM, FOS, CAZ, IMP, MEM, CRO, CTX, PIP, and AMP. Notable exceptions included SXT (10%), LEV (22%), and MIN (23%), although resistance also began to take place, (see Figure 3).

Twenty-five *Stenotrophomonas* spp. strains (47.2%) developed extensive drug resistance, 16 strains (30.2%) had resistance against less than three antibiotic agents, 11 strains (20.8%) had multiple drug resistance, and one strain (1.9%) had pandrug resistance.

Discussion

Distribution of emerging and reemerging bacteria

Acinetobacter spp. had the highest prevalence amongst the found bacteria in this study, accounted for 205/432 (47.5%) strains isolated. They are common nosocomial pathogens, associated with 26.5 to 91% morbidity and mortality rate in immunocompromised patients [4]. The respiratory tract is the most common infection site especially in patients having respiratory intubation and mechanical ventilation [5], which explains high prevalence of respiratory specimens (57.6%) in the Department of Resuscitation (50.2%). Uwingabiye et al. (2016) reported 44.67% of isolates from broncho-pulmonary samples, 54.9% isolates from the intensive care units, and 64.8% bronchopulmonary isolates in intensive care units [4]. Odsbu et al. (2017) observed 50.7% of isolates from the respiratory system [6]. Aboshakwa et al. (2019) found 70% and 33% of positive cultures from tracheal aspirate in 2012 and 2016, respectively [7]. Blood samples were also a significant source, albeit in a much less frequency (21.5%), which was too similar to other works [4].

Similar to *Acinetobacter* spp., *Klebsiella* spp. are frequent causes of hospital-acquired infections (HAI), including pneumonia, septicemia, wound infection, and meningitis, especially in neonatal intensive care patients [8]. *Klebsiella* spp. were also found at significant frequency (154 strains, 35.6%) and had

a strong occurrence in blood samples (43.5%). Bloodstream infection is, in fact, a typical result of *Klebsiella* infections with the mortality rate that may reach 79% [9].

Stenotrophomonas spp. also had a place (53 strains, 12.3%) albeit at less frequency than the above two. Although used to be rarely detected, they have been emerging as an essential nosocomial pathogen in large hospitals; the drug-resistant *S. maltophilia* is widely distributed in nature and accounted for 5.1 – 6.8% of the causes of hospital-acquired infection in the US from 2015 to 2017 [10,11,12]. The most frequent *Stenotrophomonas* spp. infections included pneumonia and bloodstream infection [13]. In our study, the bacteria were mostly isolated from blood specimens (69.8%), which was compatible with other studies where bloodstream infections were frequently observed [14].

The occurrence of *Aeromonas* spp. and *Achromonas* spp. was low (3.2% and 1.4%, respectively). They have long been recognized as pathogens in humans and animals, but many of their aspects remain poorly understood [15,16].

Antibiotics resistance of emerging and reemerging bacteria

Acinetobacter spp. have long been known for their notorious multidrug resistance and are amongst the most difficult Gram-negative pathogens to deal with. Their resistance against the recommended carbapenems has increased significantly. Odsbu et al. (2018) reported over 89% MDR rate from 2011 to 2014 [6]. In our study, the isolated *Acinetobacter* spp. expressed strong resistance against a wide range of antimicrobial agents as nearly three-quarters of the strains developed at least MDR capability, and the resistant rate was at least 66% in 17/20 agents, reaching up to 98% in aztreonam. Notable exceptions included colistin (18% R) and amikacin (24% R), two of the “old” antibiotics brought back to deal with the stubborn bacteria. The colistin resistance is unfavorable for *Acinetobacter* spp. as it requires mutation of three genes, which completely impairs lipopolysaccharide production. However, resistance against these agents has also begun to emerge [7, 17, 18].

Klebsiella spp. isolates in our study were still relatively susceptible to many antibiotics as the resistance rate was less than 50% in 10/15 agents, especially for aminoglycosides and carbapenems. The highest resistance rate was seen against ceftriaxone, aztreonam, and cefuroxime (67.4 – 72.4%). Naturally, *Klebsiella* spp. are highly sensitive to a wide range of antibiotics with exceptions of the ones naturally resisted by the Enterobacteriaceae family [19], which explains their resistance against ceftriaxone, aztreonam, and cefuroxime. Tests of isolates from patient samples also show their general susceptibility [9]. However, there are concerns of emerging Extended Spectrum β Lactamase (ESBL) producing strains resistant to penicillins, cephalosporins, and monobactams, [8,20] and carbapenemase-producing (KPC) carbapenems-resistant strains which were responsible for many hospital outbreaks [21,22], however in our study, carbapenems resistance was low in general. MDR or above was observed in 61.0% of the strains in our study. Ferreira et al. (2016) reported 84% MDR in the isolates, however, the number of isolates was small (n = 21) and the sampled population only included intensive care units [23]. *Stenotrophomonas* spp. in our study were highly resistant against testing antibiotics with resistance rate at least 70% in 16/21 agents, at least 90% in 9/21 agents, and reached 100%

in piperacillin and ampicillin. XDR was developed in 40.9% of the strains, much higher than *Acinetobacter* spp. (24.4%) and *Klebsiella* spp. (18.2%). *Stenotrophomonas* spp. are recorded to be resistant against a wide array of common antimicrobials including many penicillins, cephalosporins, aminoglycosides, macrolides, and tetracyclines, but are susceptible to minocycline, the fluoroquinolones, and polymyxins, and SXT [20], which explains the low resistance against SXT, levofloxacin, and minocycline in this study (10 – 23 %). The resistance of *Stenotrophomonas* spp. against, for example, SMX/TMP is emerging due to pronounced use of antibiotics, though, and is a significant concern for clinicians [10].

Remarks and recommendations

Antibiotics resistance is a concerning issue in healthcare. In our study, we observed significant drug resistance in the investigated bacteria, especially *Acinetobacter* spp. and *Stenotrophomonas* spp. The resistance capability, nonetheless, was within expectation from the medical literature. *Acinetobacter* spp. were still vulnerable to colistin and amikacin, *Stenotrophomonas* spp. still did not resist well against minocycline, levofloxacin, and SXT, and *Klebsiella* spp. were susceptible to a wide range of antibiotics and strong resistance against carbapenems was not spotted. The threat of resistance against them, however, still cannot be ignored since resisting strains have been emerging as seen in the ESBL and KPC. Close monitoring of antibiotics resistance, therefore, is critical, and the rapid detection methods for resistant strains [18, 24] can be very helpful. There has been significant development in insights into the molecular and physiologic mechanism of antibiotic resistance of the studied bacteria [8, 12, 20] that may lay the basis for the development of countermeasures.

Many emerging and re-emerging bacteria including the ones in this study are notable nosocomial and opportunistic pathogens whose outbreaks and infections have a considerable association with medical procedures and apparatus, such as invasive surgeries, immunocompromised-related cancer therapies, and transplants, invasive equipment such as catheters and ventilators, and operations in intensive care and resuscitation units [6,7,10,14]. Exchanges of the patients, moreover, are important lines for drug resistance spreading [21]. Therefore, prevention and control of hospital-acquired infections are essential and require multiple efforts from many aspects of the system, including management and organization, and the awareness of the staff and patients. However, such procedures can be a tough challenge in developing countries which have limited resources [22]. In many cases, general knowledge or practices of medical staffs of HAI preventions and guidelines are not perfect [25] which reveals the needs to improve professional education and training for the staffs, together with increase cooperation, supervision and feedback from the hospital units and other stakeholders for better hygiene practices.

Conclusions

There were 432 bacterial strains from five genera isolated from the medical specimens in 7A Military Hospital from January 2017 to June 2019 with increasing isolates each year. *Acinetobacter* spp., *Klebsiella* spp., and *Stenotrophomonas* spp. are predominant amongst the isolates. *Acinetobacter* spp. was mostly seen in the Department of Resuscitation, *Klebsiella* spp. in

the Department of Infectious Diseases, and *Stenotrophomonas* spp. in the Department of Internal Medicine. Antibiotic resistance was significant, especially in *Acinetobacter* spp. and *Stenotrophomonas* spp., but within literature expectation. Close attention to emerging resistant strains and HAI prevention is essential to prevent the risks of resistant emerging and re-emerging bacteria.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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