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*Original Article*

# Prevalence of multidrug resistant Gram-positive cocci in a Chinese hospital over an 8-year period

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**Abstract:** Gram-positive cocci are common causes of bloodstream and nosocomial infections, and their multi-drug resistance is an increasingly serious problem. The present study aimed to assess the prevalence of multi-drug-resistant Gram-positive cocci in a Chinese population. In this retrospective study, data about Gram-positive cocci from in-patients (January 2006 and December 2013) at the Second Hospital of Shanxi Medical University, Taiyuan, China, were reviewed. Antimicrobial susceptibility profile of the isolated Gram-positive cocci was evaluated using the disk diffusion method. Antibiotic resistance was determined according to the Clinical and Laboratory Standards Institute 2009 guidelines. The prevalence of drug resistance was determined, as well as correlation coefficients for various drugs between the resistance rate and year of sample collection. A total of 7789 Gram-positive cocci isolates were found, including 2576 (33%) coagulase-negative *Staphylococci*, 1477 (19%) *Staphylococci aureus*, 1343 (17%) *Enterococcus faecalis*, and 1139 (15%) *Enterococcus faecium*. The proportions of methicillin-resistant *Staphylococci aureus* (MRSA) and methicillin-resistant *Staphylococci* (MRS) were 31.5% (465/1477) and 61.6% (1587/2576), respectively. Among all isolates, MRS had much higher drug resistance rate than methicillin-sensitive *Staphylococci* (P<0.05). *E. faecalis* had a higher multi-drug resistance rate than *E. faecium* (P<0.01). Interestingly, MRSA resistance rates declined over the years, showing a negative correlation coefficient for all drugs, with significance for levofloxacin, azithromycin, erythromycin, and clindamycin (P<0.05), but not sulphamethoxazole/trimethoprim (P=0.057) and gentamicin (P=0.186). These results indicated that *Staphylococci* were the predominant Gram-positive cocci isolated. There was a trend of decreasing MRSA in the population studied.

**Keywords:** Antimicrobial resistance, Gram-positive cocci, multidrug resistant *Staphylococcus aureus*, decreasing resistance

## Introduction

Gram-positive bacteria are common causes of bloodstream and nosocomial infections in the USA, and there is an increasing trend of infections caused by antibiotic-resistant Gram-positive bacteria [1]. Drug-resistant strains of Gram-positive cocci have attracted increasing attention around the globe. Indeed, infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and penicillin-resistant *Streptococcus pneumoniae* were reported to be continuously rising in different countries [2-4]. In the USA, approximately 60% of intensive care unit (ICU)-diagnosed staphylococcal infections are caused by MRSA, with an increasing incidence [1].

Vancomycin is the standard antibiotic treatment for serious MRSA infections, but cases of vancomycin-resistant *Staphylococcus aureus* have recently emerged in the USA [1]. The incidence of VRE continues to escalate in hospitals, with the overwhelming majority of infections due to *Enterococcus faecium* (*E. faecium*) [1]. Multidrug-resistant coagulase-negative staphylococci are a major cause of nosocomial bloodstream infections, especially in critically ill and hematology patients, and glycopeptide antibiotics have been considered the drugs of choice [5]. A recent study on the antibiotic susceptibility of *Staphylococcus epidermidis*, *Streptococcus mitis*, and *E. faecium* clinical isolates to erythromycin and spiramycin (macrolides), clindamycin (lincosamide), and pristinamycin (streptogramin) indicated that the iso-

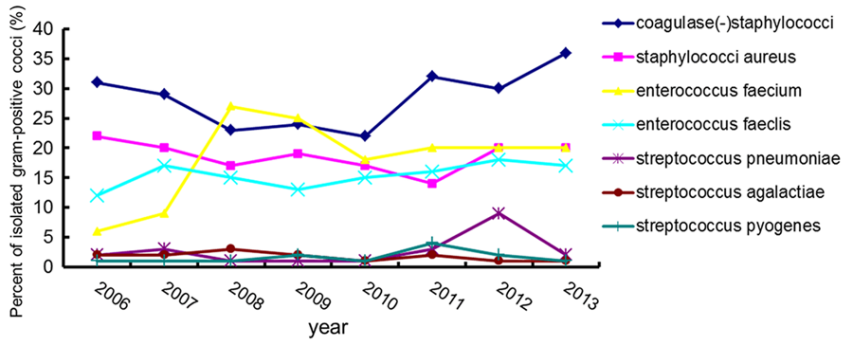


**Figure 1.** Trend of Gram-positive cocci isolated between 2006 and 2013.

lates were mostly resistant to macrolides and lincosamide, but usually susceptible to pristinamycin [6]. The emergence of glycopeptide-resistant *Staphylococcus aureus* in Japan [4] and the USA [7], and the heteroresistance to glycopeptides of MRSA [8, 9] have raised the concern of the limited therapeutic options for these Gram-positive multi-drug-resistant bacteria that largely cause nosocomial infections.

It was recently suggested that the Chinese healthcare system has a limited application of antimicrobial stewardship, encouraging over-prescribing and self-medication, which lead to high rates of resistance among bacteria, tubercle bacillus, and fungi [10]. Hence, in the present study, Gram-positive coccus isolate data (from January 2006 to December 2013) were retrospectively analyzed to understand the drug sensitivities of these isolates. In addition, the prevalence of resistance was determined by linear regression to obtain correlation coefficients for the various drugs studied, between resistance and year of sample collection. Interestingly, we found a decreasing trend of MRSA over the years in the population studied. These findings stress the importance of regulating antibiotic use, providing additional tools to clinicians for selecting appropriate antibiotics.

## Materials and methods

### *Isolated strains*

The present study was approved by the Research Ethics Committee of The Second Hospital of Shanxi Medical University.

Specimens were collected from seven anatomical sites including nostrils, oropharynx, axilla, mouth, web spaces of the dominant hand, web spaces of the foot, and perirectal area from in-patients hospitalized at internal medicine departments (Department of respiration, Department of hematology, Department of neurology, Department of nephrology, and Department of Gastroenterology), surgery departments (Department of general surgery, Department of thoracic surgery, Department of Neurosurgery, and Department of Urology) and ICU between January 2006 and December 2013 at The Second Hospital of Shanxi Medical University, Taiyuan, China. Only the first Gram-positive cocci isolate from each patient was used. The study was approved by the ethics committee of The Second Clinical Medical College. All specimens were collected after patients provided written informed consent.

Immediately after specimen collection using pre-moistened swabs (Copan Liquid Stuart media culture, Copan Inc., Italy), the samples were transported to the laboratory and plated onto Trypticase Soy Agar with 5% sheep blood (blood agar plate). Specimens were also cultured on CHROMagar™ *S. aureus* plates for rapid detection of *S. aureus*. After incubation at 35°C, colonies morphologically consistent with *S. aureus* on blood agar plates and mauve large colonies on CHROMagar™ *S. aureus* plates were subcultured on blood agar plates in order to ensure culture purity. All isolates were frozen at -80°C in Trypticase Soy Broth with 15% glycerol.

### *Susceptibility testing*

The antimicrobial susceptibility profiles of the isolated Gram-positive cocci were assessed using the disk diffusion method. Multiple antimicrobial agents including vancomycin (30 μg), teicoplanin (30 μg), ampicillin (10 μg), penicillin (10 U), gentamicin (120 μg and 10 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), azithromycin (15 μg), amoxicillin/clavulanic acid (30 μg), levofloxacin (5 μg), clindamycin (2 μg), fosfomy-

**Table 1.** The isolated MRSA, MSSA, MRCNS, and MSCNS strains and their resistance to 14 antibiotics

| Antibiotics | MRSA | | | MSSA | | | | MRCNS | | | MSCNS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Number of isolates | %R | %I | Number of isolates | %R | %I | P | Number of isolates | %R | %I | Number of isolates | %R | %I% | P |
| Penicillin G | 210 | 100 | 0 | 464 | 94.8 | 0 | 0.001 | 496 | 100 | 0 | 470 | 91.8 | 0 | <0.001 |
| Azithromycin | 83 | 100 | 0 | 163 | 74.8 | 14.1 | <0.001 | 147 | 98 | 2 | 136 | 88 | 2.7 | 0.001 |
| Ciprofloxacin | 111 | 97.1 | 0 | 273 | 12.1 | 9.2 | <0.001 | 316 | 86.6 | 4.5 | 215 | 60.4 | 7.7 | 0.001 |
| Amoxycillin/clavulanic | 161 | 92.9 | 0 | 419 | 25.3 | 0 | <0.001 | 451 | 79.4 | 0 | 327 | 25.3 | 0 | <0.001 |
| Gentamicin | 188 | 91.7 | 1.8 | 390 | 29.7 | 2.3 | <0.001 | 446 | 76.9 | 2.2 | 339 | 43.7 | 2.3 | <0.001 |
| Erythromycin | 131 | 91.3 | 5.6 | 339 | 69.6 | 17.4 | <0.001 | 417 | 95 | 2.5 | 290 | 87 | 2.5 | <0.001 |
| Levofloxacin | 100 | 90.6 | 0 | 268 | 11.6 | 1.9 | <0.001 | 277 | 75 | 7.6 | 260 | 44.8 | 17 | <0.001 |
| Clindamycin | 197 | 89.1 | 2.3 | 444 | 47.3 | 18.7 | <0.001 | 499 | 72.2 | 10.8 | 364 | 53 | 14 | <0.001 |
| Fosfomycin | 202 | 78.7 | 2.8 | 432 | 2.9 | 1.2 | <0.001 | 485 | 61.3 | 8.4 | 365 | 8.5 | 1 | <0.001 |
| Tetracycline | 148 | 75.8 | 4.8 | 325 | 20.6 | 3.4 | <0.001 | 389 | 36.3 | 0.5 | 246 | 38.8 | 3.1 | 0.547 |
| Sulphamethoxazole/trimethoprim | 191 | 26.5 | 11.2 | 432 | 24.3 | 2.3 | 0.524 | 473 | 82.4 | 3.8 | 368 | 75.3 | 2.1 | 0.035 |
| Vancomycin | 218 | 0 | 0 | 463 | 0 | 0 | -- | 502 | 0 | 0 | 377 | 0 | 0 | -- |
| Teicoplanin | 151 | 0 | 0 | 340 | 0 | 0 | -- | 377 | 0 | 0 | 350 | 0 | 0 | --- |
| Linezolid | 96 | 0 | 0 | 191 | 0 | 0 | -- | 216 | 0 | 0 | 323 | 0 | 0 | --- |

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRCNS, methicillin-resistant coagulase-negative *staphylococcus*; MSCNS, methicillin-sensitive coagulase-negative *staphylococcus*; %R: resistance rate; %I: intermediary rate.

**Table 2.** Resistance of *Enterococcus faecium* and *Enterococcus faecalis* towards 15 different antibiotics

| Antibiotics | *Enterococcus faecalis* | | | *Enterococcus faecium* | | | *P* value |
|---|---|---|---|---|---|---|---|
| | Number of isolates | %R | %I | Number of isolates | %R | %I | |
| Penicillin G | 574 | 25.3 | 0 | 662 | 94.3 | 0 | <0.001 |
| Vancomycin | 564 | 0 | 0 | 662 | 0 | 0.3 | -- |
| Gentamicin (120 μg) | 556 | 42.4 | 1.1 | 628 | 89.6 | 0.5 | <0.001 |
| Ampicillin | 536 | 26.3 | 0 | 552 | 92.2 | 0 | <0.001 |
| Rifampicin | 489 | 47 | 14.1 | 592 | 80.2 | 9.3 | <0.001 |
| Levofloxacin | 485 | 45.6 | 10.7 | 589 | 93.7 | 3.1 | <0.001 |
| Teicoplanin | 418 | 0 | 0 | 546 | 0 | 0.2 | -- |
| Piperacillin | 348 | 16.7 | 6.9 | 500 | 96.2 | 0.4 | <0.001 |
| Minocycline | 325 | 31.7 | 32 | 434 | 21.4 | 18.4 | 0.001 |
| Tetracycline | 236 | 58.9 | 7.2 | 287 | 45.3 | 1.7 | 0.002 |
| Linezolid | 232 | 0 | 0 | 342 | 0 | 0 | -- |
| Norfloxacin | 197 | 62.9 | 19.3 | 167 | 94.6 | 3 | <0.001 |
| Erythromycin | 168 | 89.3 | 8.3 | 140 | 96.4 | 2.1 | 0.018 |
| Nitrofurantoin | 98 | 18.4 | 6.1 | 102 | 54.9 | 5.9 | <0.001 |
| Chloramphenicol | 197 | 25.9 | 6.1 | 128 | 14.8 | 10.9 | 0.018 |

%R: resistance rate; %I: intermediary rate.

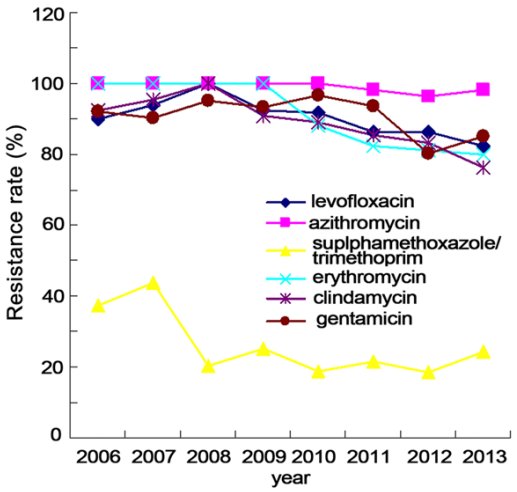


**Figure 2.** Annual antimicrobial resistance rates of MRSA toward selected antibiotics.

cin (50 μg), sulfamethoxazole/trimethoprim (25 μg), rifampicin (5 μg), linezolid (30 μg), piperacillin (100 μg), minocycline (30 μg), nitrofurantoin (300 μg), and erythromycin (15 μg) were used. Culture media were obtained from Oxoid Ltd. (Basingstoke, UK). The broth dilution method was used to determine the minimal inhibitory concentration (MIC) for each vancomycin-resistant isolate. All susceptibility tests were performed and interpreted according to guidelines established by the Clinical and Laboratory Standards Institute, 2009 [4]. *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *E. Faecalis* ATCC 29212 obtained from the National Center for Clinical Laboratories in China were used as controls.

*MRSA resistance rates*

MRSA resistance rates were determined for various antibiotics including levofloxacin, azithromycin, erythromycin, clindamycin, sulfamethoxazole/trimethoprim, and gentamicin (P=0.186), and expressed in percentage (resistance prevalence) for each year of sample collection.

*Statistical methods*

All raw data were analyzed using the WHONET software, version 5.4 (WHO Antimicrobial Resistance Monitoring system). All statistical analyses were performed using SPSS 18.0 (IBM, Armonk, NY, USA). Frequencies were compared using the Chi-square test. Linear regression was used to determine the correlation between MRSA resistance rate and year of sample collection to observe if a trend could be observed between resistance rates and time, using years as the independent variable and resistance rate as the dependent variable. Regression equations were derived for each drug. P<0.05 was considered statistically significant.

## Results

*Gram-positive cocci isolated in 2006-2013*

Between January 2006 and December 2013, 7789 Gram-positive bacterial strains were isolated including coagulase-negative *staphylococci* (n=2576, 33%), *S. aureus* (n=1477, 19%), *E. faecium* (n=1343, 17%), and *E. faecalis* (n=1139, 15%). As shown in **Figure 1**, a high number of coagulase-negative staphylococci were isolated, with an increasing trend from 2011 whereas *S. aureus* prevalence decreased. The prevalence of *E. faecalis* increased during

**Table 3.** Correlation analysis between MRSA resistance rate and year of samplecollection for various antibiotics

| Antibiotic | Regression equation | R2 | α | β | r (correlation coefficient) | P |
|---|---|---|---|---|---|---|
| Levofloxacin | y=-1.601x+3307.905 | 0.512 | 3307.905 | -1.601 | -0.715 | 0.046* |
| Azithromycin | y=-0.435x+972.263 | 0.587 | 972.263 | -0.435 | -0.766 | 0.027* |
| Sulphamethoxazole/trimethoprim | y=-2.630x+5310.744 | 0.480 | 5310.744 | -2.630 | -0.693 | 0.057 |
| Erythromycin | y=-3.560x+7244.313 | 0.853 | 7244.313 | -3.560 | -0.924 | 0.001* |
| Clindamycin | y=-2.624x+5361.595 | 0.747 | 5361.595 | -2.624 | -0.865 | 0.006* |
| Gentamicin | y=-1.175x+2451.900 | 0.271 | 2451.900 | -1.175 | -0.521 | 0.186 |

Linear regression was used to determine the correlation between MRSA resistancerate and year of sample collection, with regression equations derived for each drug.
*Indicates a significant correlation.

the 8 years, peaking in 2008 (27%). The prevalence of *E. faecium* was gradually increased in the last few years, while *Streptococci* isolate numbers did not show a marked change and remained lower than 5%.

*Antimicrobial resistance of staphylococci*

Isolates from various time points did not demonstrate resistance or increasing resistance in vancomycin and teicoplanin MICs. The frequencies of MRSA and methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS) isolates were 31.5% (287/912) and 61.6% (1080/1754), respectively. MRS strains showed much higher resistance rates than methicillin-sensitive staphylococcus (MSS) strains ($P< 0.05$, **Table 1**).

*Antimicrobial resistance of enterococci*

The resistance rate of *E. faecium* to chloramphenicol and tetracycline was much higher than that of *E. faecalis*. However, *E. faecium* resistance to other antibiotics was less pronounced than that of *E. faecalis*. There was no resistance observed toward teicoplanin or vancomycin from either enterococci (**Table 2**).

*Antimicrobial resistance rate of MRSA throughout the years*

MRSA resistance varied for the antibiotics tested (**Figure 2**), but showed a generally decreasing trend. Among these drugs, the sulfamethoxazole/trimethoprim combination was the most effective regimen against MRSA throughout the 8 years of study (resistance rates decreasing from 37.5% to 18.4 between 2006 and 2013). MRSA showed the highest resistance to azithromycin (from 100% in 2006 to 98.2% in 2013). The other antibiotics studied (erythromycin, levofloxacin, clindamycin, and gentamicin) had intermediate effects and showed higher activity during the last 2 years in comparison with the previous time points.

Next, we evaluated the relationship between MRSA resistance rate (prevalence) and year of sample collection, and equations were derived by linear regression for each drug studied. Interestingly, a negative correlation was obtained for all antibiotics, as shown in **Table 3**. For most of them, a significant correlation was obtained: levofloxacin, r=-0.715, P=0.046; azithromycin, r=-0.766, P=0.027; erythromycin, r=-0.924, P=001; clindamycin, r=-0.865; P=0.006 (**Table 3**). A non-significant correlation was obtained for sulfamethoxazole/trimethoprim (r=-0.693, P=0.057) and gentamicin (r=-0.521, P=0.186) between resistance rate and year (**Table 3**). These results suggest that the rate of resistance to these antibiotics decreased in recent years.

## Discussion

In the past 20 years, Gram-positive cocci have been considered the major emerging pathogens causing nosocomial infections. The present study demonstrated that coagulase-negative *Staphylococcus* isolates had a higher prevalence compared with enterococci. *Streptococcus* isolates were found at a prevalence <5%. *Staphylococcus* is an important emerging pathogen that causes several diseases including skin and wound infections, bacteremia, and necrotizing pneumonia. In addition, multiple studies have demonstrated that *Staphylococcus* has always been an important pathogen causing Gram-positive cocci-related nosocomial infections [2, 11-13]. The epidemiological characteristics of *S. aureus*, especially MRSA, are changing rapidly. Methicillin, the first penicillinase-resistant penicillin, revolutionized the treatment of penicillin-resistant *S. aureus* when

introduced into clinical practice in 1959 [14]. Within just two years, however, methicillin-resistant strains began to emerge. During the following five decades, MRS strains continued to cause nosocomial infections worldwide [15]. The analysis of the resistance of Gram-positive cocci in ten Chinese teaching hospitals by Sun et al. [16] in 2007 revealed that among the MRS strains, *S. aureus* (40.5%) was predominantly found, representing 84.8% of coagulase-negative *Staphylococcus*. In the present study, over a period of 8 years, the isolated MRSA frequency was between 29.3 and 34.6%, while the MRCNS frequency was between 56.8 and 67.7%; both were lower than the national average. In addition, no vancomycin-, teicoplanin-, or linezolid-resistant *Staphylococcus* strains were found. The MRS isolates showed much higher antibiotic resistance than MSS isolates ($P<0.05$). Hence, while treating coccus infections, clinicians should define clearly whether they are methicillin-resistant. However, both MSSA and MRSA showed no significant difference in the resistance rate toward sulfanomides ($P>0.05$), suggesting that clinicians can use sulfanomides as empirical therapy for uncharacterized *S. aureus* infections. Similarly, MSCNS and MRCNS had no significant difference in the resistance rate toward tetracyclines ($P=0.0547$), making these antibiotics the empirical therapy of choice. The MRSA isolates had decreased resistance to sulfanomides, vancomycin, teicoplanin, and linezolid, with 26.5%, 0%, 0%, and 0% resistance rates, respectively. However, the MRSA strains showed multiple drug resistance to the other commonly used antibiotics with a resistance rate greater than 75%. The MSSA strains were very susceptible to commonly used antibiotics, showing a resistance rate of less than 30%. However, they showed high resistance toward penicillins, macrolides, and clindamycin. Therefore, these antibiotics may be used as empirical therapy for MSSA infections.

Enterococci are widespread in nature, causing various diseases such as urinary tract infections, intra-abdominal abscesses, wound infections, endocarditis, and bacteremia [17]. Among the 30 species of the genus *Enterococcus*, *E. faecalis* and *E. faecium* are the most common species infectious to humans [18]. In the present study, *E. faecalis* prevalence increased between 2006 and 2013, peaking in 2008 (27%) and declining in later years, although it remained significantly higher than the 2006 value. This 3% increase over the 8 years might be explained by the overuse of third generation cephalosporins before 2007 as drugs of choice [19, 20]. In the recent two years, due to more strict regulations, clinicians rationalized the use of antibiotics based on microbiological test reports. Indeed, the use of third generation cephalosporins had declined significantly, which was followed by decreased prevalence of resistant enterococci isolates. In a study conducted in a Greek hospital, the authors reported an eight-fold increase [21] in *E. faecium* infection incidence. The enterococci have a complex drug resistance mechanism owing to their thick cell wall [22]. Antimicrobial resistance can be divided into natural resistance, acquired resistance, or tolerance [23]. In the present study, among the 14 antibiotics tested, *E. faecium* showed significantly higher resistance compared with *E. faecalis* ($P<0.001$). Therefore, they should be differentiated in the clinical setting while selecting the appropriate antibiotics. The VRE isolates were first recognized in the 1980s in Europe and the USA [24]. Their prevalence has been increasingly rising across the globe, and China is no exception [25]. Interestingly, we showed here that none of the isolated enterococci strains was resistant to teicoplanin, vancomycin, or linezolid. This lack of resistance is intriguing for an inland city like Taiyuan. Indeed, it is generally accepted that antibiotic abuse is particularly severe in small- and medium-sized cities and rural areas where doctors are not as well educated as in the large cities [26].

Next, we analyzed the MRSA prevalence trend in time. Interestingly, a negative correlation was obtained for all the antibiotics assessed, indicating a decrease in MRSA resistance with time in this study population. Importantly, this correlation was significant for levofloxacin, azithromycin, erythromycin, and clindamycin. These encouraging findings confirm that MRSA resistance can be reduced if appropriate measures and regulations are put into place for optimal antibiotic use [27]. Indeed, decreases in resistance rates mostly occurred in the last years of our survey, likely due to increasing awareness and new regulations from Chinese health authorities.

A few limitations of this study should be mentioned. It was a monocentric study and the test-

ed population belonged to the same ethnicity. Including multiple institutes/hospitals would have benefited the study.

In conclusion, among the isolated Gram-positive cocci, *Staphylococcus* was the most frequent pathogen causing nosocomial infections in the last 8 years in this specific population of Chinese in-patients. Importantly, MRSA resistance rates declined over the years, especially in the last ones. These findings indicate that continuous monitoring of antibiotic sensitivity and rationalizing the use of antibiotics remain an important and effective strategy to minimize the emergence of multiple resistance strains.

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## Disclosure of conflict of interest

None.

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