In vitro activities of vancomycin and linezolid against biofilm-producing methicillin-resistant staphylococci species isolated from catheter-related bloodstream infections from an Egyptian tertiary hospital

Asmaa A. Hashem,1 Noha M. Abd El Fadeal2 and Atef S. Shehata1,3,*

Abstract

Purpose. Catheter-related bloodstream infections (CRBSIs) are among the most common hospital-acquired infections. We aimed to survey methicillin resistance, biofilm production and susceptibility to vancomycin, linezolid and other antibiotics for staphylococci isolated from CRBSIs.

Methodology. Fifty-eight isolates [20 S. aureus and 38 coagulase-negative staphylococci (CoNS; 20 Staphylococcus epidermidis, nine Staphylococcus haemolyticus, three Staphylococcus schleiferi, two Staphylococcus warneri and four Staphylococcus lugdunensis)] were tested for methicillin resistance by cefoxitin disk diffusion and detection of the meca gene by PCR; biofilm-forming ability using Congo red agar and tissue culture plate methods; susceptibility to ciprofloxacin, clindamycin, cotrimoxazole, erythromycin, gentamicin, linezolid, rifampicin and tetracycline; and MIC determination for vancomycin.

Results/Key findings. Cefoxitin resistance was detected among 40 % (8/20) S. aureus isolates, 70 % (14/20) S. epidermidis isolates and 16.7 % (3/18) of other CoNS, although the meca gene was detected in 45 % (9/20) S. aureus isolates, 35 % (7/20) S. epidermidis isolates and 16.7 % (3/18) of other CoNS. Biofilm-forming ability ranged from 45 to 75 %. Methicillin-resistant S. aureus and other CoNS were considered to be more virulent than methicillin-resistant S. epidermidis due to the higher biofilm forming abilities of the former. All tested isolates exhibited 100 % sensitivity to vancomycin and linezolid, irrespective of their methicillin resistance or biofilm-forming ability. Rifampicin showed overall sensitivity of 75.9 %. Varying degrees of multi-resistance were found for the other antibiotics.

Conclusion. Vancomycin, linezolid and rifampicin could be used effectively against methicillin-resistant staphylococci isolated from CRBSIs.

INTRODUCTION

Catheter-related bloodstream infection (CRBSI) is defined as bacteraemia attributed to the presence of an inserted venous catheter [1]. Although venous catheters, either central or peripheral, are mandatory and sometimes lifesaving tools for patients in hospitals, especially in intensive care units, they can lead to CRBSIs [2]. These infections are among the most lethal, debilitating and costly hospital-acquired infections, cause major problems in hospital environments and impose a great burden on healthcare costs [2, 3]. Indeed, CRBSIs are major leading causes of mortality and morbidity in hospitalized critically ill and debilitated patients [4–6].

CRBSIs are usually caused by bacteria present in the resident skin flora at venous catheter sites, and staphylococci appear on top of these bacterial causes [7, 8]. Staphylococcus aureus and coagulase-negative staphylococci (CoNS) are the most commonly identified major causes of CRBSIs [7, 9]. Their incidence varies greatly from one study to another, as the detection rate was 40 % for S. aureus [10] while the rates
for CoNS were 50 and 14 % in two different studies [11, 12], respectively. Antibiotic resistance and biofilm formation are cornerstone virulence factors helping these bacteria cause CRBSIs that are resistant to treatment.

Most of the staphylococci species isolated from CRBSIs are methicillin-resistant [13–15]. Although resistance to methicillin can be caused by hyperproduction of β-lactamase enzymes, the production of modified penicillin-binding protein, PBP2a, encoded by the meca gene, is the major mechanism underlying this resistance [16, 17]. Here, the use of PCR to detect this gene is of great help in detecting such methicillin-resistant bacteria.

Different species of staphylococci have the ability to form biofilms on intravenous catheters, these consisting of bacterial communities intermingled with the extracellular matrix. Such biofilms act as principal foci and sources for continual shedding of bacteria into blood with subsequent bacteraemia, in addition to interference with immunologic defences and marked resistance to antimicrobials [8]. Many studies have found that the sensitivities of methicillin-resistant S. aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) to a wide array of antibiotics have decreased after the development of biofilms by such bacteria [18].

Vancomycin was used for a considerable period of time as a standard antibiotic against methicillin-resistant staphylococci, but with the development of resistance against it, many other antibiotics including linezolid, tigecycline and daptomycin now have major roles in treating CRBSIs caused by MRSA and MRCoNS. Unfortunately, different levels of resistance have developed against all of these drugs [19] and, hence, antimicrobial susceptibility testing for staphylococci isolated from CRBSI cases is mandatory for accurate selection of the antibiotics required to achieve proper management of these infections. Our study aimed to survey methicillin resistance and the biofilm-production ability of staphylococci species isolated from CRBSI cases in a tertiary hospital, as well as testing their in vitro susceptibility patterns for vancomycin and linezolid to investigate whether these patterns can be affected by methicillin resistance and biofilm-forming ability.

METHODS

Study subjects

A total of 150 patients were diagnosed as having a CRBSI [after their admission to the intensive care unit (ICU), neonatal ICU, cardiothoracic surgery ICU and haemodialysis unit at Suez Canal University Hospital in the period from January 2015 to December 2015] according to clinical and laboratory findings [20], and according to the Centers for Disease Control and Prevention guidelines [21]. CRBSI was identified as bacteraemia by one positive blood culture when the catheter-segment culture yielded more than 15 c.f.u. of the same organism and no other source of infection was identifiable.

Collection and processing of specimens

From each patient with an episode of CRBSI, two specimens (catheter tip specimen and blood specimen for blood culture) were concomitantly aseptically collected, processed for isolation and the causative organisms identified.

Semi-quantitative catheter segment culture

A 5 cm segment from each withdrawn catheter was rolled four times across a blood agar plate with firm downward pressure [22]. Cultures that yielded 15 or more colonies were considered significant, potentially indicating a catheter-related infection.

Blood specimens

Blood samples were collected before initiation of antimicrobial treatment. Blood (5 ml) was drawn under aseptic conditions and added to a monophasic culture bottle (BacT/ALERT FA FAN Aerobic or BacT/ALERT PF Pediatric FAN; bioMerieux), incubated at 37 °C and examined daily for up to 7 days. Subcultures of blood culture broth were added to blood agar and MacConkey’s agar and incubated aerobically at 37 °C.

Identification of Staphylococcus species causing CRBSI

Isolated colonies were identified by colony morphology, Gram-staining and biochemical tests. For Gram-positive bacteria, catalase, slide and tube coagulase, and mannitol, fermentation tests were used. CoNS species were determined by testing novobiocin sensitivity, urease, D-mannose acid production and ornithine decarboxylase [23].

Phenotypic characterization of methicillin resistance

All Staphylococcus species were phenotypically tested for methicillin resistance using the disk diffusion method with a cefoxitin disk (30 µg). Interpretation of the inhibition zone diameter was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [24]; any growth of S. aureus isolates around the disk ≤21 mm and any growth of CoNS isolates ≤24 mm were considered as resistance.

Genotypic characterization of methicillin-resistant Staphylococcus species

Bacterial DNA extraction

Genomic DNA was extracted using the boiling method as described previously [25] with slight modifications. Briefly, several colonies from an overnight-grown culture on nutrient agar were resuspended in 1 ml PBS and centrifuged at 12 000 g for 5 min. The supernatant was discarded and the pellet resuspended in 100 µl TE, placed in a boiling water bath for 10 min and transferred to ice. This suspension was stored at −20 °C until used later as a direct source of template DNA.

Detection of meca gene by PCR

Amplification of the meca gene (310 bp) was carried out by PCR as previously mentioned [26] with some modifications. We used the forward primer 5’-GTA GAA ATG ACT GAA
CGT CCG ATA A-3’ and the reverse primer 5’-CCA ATT CCA CAT TGT TTC GGT CTA A-3’ which are described in another study [27]. The 25 μl reaction volume contained 1 μl each of the forward and reverse primers (1 μM each), 5 μl of the extracted DNA, 12.5 μl of PCR master mix (10 mM Tris–HCl, pH 9.0, 50 mM KCl, 0.1 % Triton x-100, 2.5 mM MgCl₂, 200 μM each of dATP, dCTP, dGTP and dTTP, 1 U Taq DNA polymerase) and 5.5 μl sterile double-distilled water. Cycling conditions were a hot start at 94 °C for 4 min followed by 30 cycles at 94 °C for 45 s, 50 °C for 45 s and 72 °C for 1 min, and a final extension step at 72 °C for 3 min. The amplifications were carried out using the Veriti thermal cycler (Applied Biosystems). One positive and two negative control reactions were included in each run. The positive control reaction contained a DNA template extracted from a clinical S. aureus isolate previously isolated and accurately identified in our laboratory as MRSA and positive for the mecA gene, while one negative control reaction contained DNA extracted from S. aureus ATCC 25923 as methicillin-sensitive S. aureus and the other negative control reaction contained sterile double-distilled water in place of the DNA template. The amplified products were tested by agarose gel electrophoresis using 2 % agarose in Tris-borate-EDTA and 100 bp DNA ladder (Cleaver Scientific), stained with ethidium bromide and visualized under a UV transilluminator. Photographs were captured using a gel documentation system (BioDocAnalyze, Biometra).

Antibiotic susceptibility testing

The susceptibilities of all isolated staphylococci were tested for ciprofloxacin (5 μg), clindamycin (2 μg), cotrimoxazole (25 μg), erythromycin (15 μg), gentamicin (10 μg), linezolid (30 μg), rifampicin (5 μg) and tetracycline (30 μg; all purchased from Oxoid) using the standard disk diffusion method outlined in the CLSI guidelines [24]. S. aureus ATCC 25923 was used as a quality control strain.

Vancomycin susceptibility was tested by determining its MIC using the agar dilution method according to CLSI M07-A9 [28] guidelines. Twofold dilutions of different concentrations of vancomycin (Sigma-Aldrich) were freshly prepared and added to Mueller–Hinton agar solid media to obtain the desired final concentrations (ranging from 0.5 to 64 mg L⁻¹). A standardized bacterial suspension of 1–5 × 10⁵ c.f.u. ml⁻¹ (from each tested isolate) was spotted on the solid media. S. aureus ATCC 29213 was used as a quality control strain.

The MIC endpoints were read after 24 h of incubation at 37 °C. The MIC values were interpreted as vancomycin-susceptible ≤2 μg ml⁻¹, intermediate susceptible 4 to 8 μg ml⁻¹ and resistant ≥16 μg ml⁻¹ for S. aureus isolates; and, for CoNS, susceptible ≤4 μg ml⁻¹, intermediate susceptible 8 to 16 μg ml⁻¹ and resistant ≥32 μg ml⁻¹.

Identification of biofilm-forming isolates

The identification of exopolysaccharide-forming Staphylococcus species isolates was carried out using both the spectrophotometric tissue-culture plate (TCP) method and Congo red agar (CRA) plate tests.

For the spectrophotometric TCP tests, according to [29–31], all Staphylococcus isolates (n=58) were inoculated into trypticase soy broth with 1 % glucose and incubated for 24 h at 37 °C, then diluted (1 : 100) with fresh medium. Aliquots of 0.2 ml of the diluted cultures were transferred to wells of sterile, polystyrene, flat-bottom 96-well tissue culture plates (Sigma-Aldrich). Wells containing plain broth only were included to serve as negative controls to check sterility. The plates were incubated for 24 h at 37 °C. Then, the contents of the wells were gently removed and the wells washed four times with 0.2 ml volumes of PBS (pH 7.2) to remove free-floating planktonic bacteria. Next, 100 μl of 1 % solution of crystal violet was added to each well. The plates were incubated at room temperature for 15 min and rinsed thoroughly and repeatedly with water. Afterwards, the stained biofilms were decolorized in 200 μl volumes of fresh 95 % ethanol, 125 μl samples of which were then transferred to the wells of a new polystyrene 96-well plate. These were then read to determine OD using a micro ELISA auto reader (STAT FAX-2100) at a wavelength of 540 nm, and the obtained values were considered as an index of bacteria adhering to the surface and forming biofilm. To compensate for background absorbance, OD readings of wells with ethanol were used as blanks and subtracted from all test values. The test was carried out in duplicate for each isolate and repeated on three different occasions, and the means of OD values were calculated.

The isolates were classified into three categories: non-adherent, moderately adherent and strongly adherent. When mean OD values ≤0.111, which was the non-adherent cut-off, the isolates were considered as negative; and when the cut-off corresponded to moderately (mean OD values >0.111 or ≤0.222) or strongly adherent (mean OD values >0.222), the isolates were considered as positive [31].

CRA plate test

CRA plates were prepared by adding 0.8 g Congo red (Loba Chemie) and 36 g saccharose (El Nasr Chemical) to 1 l of brain heart infusion agar (Lab M) and sterilizing by autoclaving. The plates were inoculated and incubated for 24 h at 37 °C. Slime-producing staphylococci grew as black colonies, while non-slime-producing strains grew as red colonies [32].

Statistical analysis

All obtained results were in the form of qualitative data expressed as categorical variables and presented in counts (numbers) and percentages for tested isolates. Chi-square or Fisher’s exact tests were utilized to test the statistical significance of the differences between the study groups. Statistical difference was considered to be significant with P values<0.05. Data entry and analysis were carried out using the Statistical Package for Social Sciences version 20.0 (IBM Corporation, NY).
RESULTS

Fifty-eight non-duplicate clinical strains were isolated and identified. These included 20 *S. aureus* and 38 isolates of CoNS (20 *S. epidermidis*, nine *S. haemolyticus*, three *S. schleiferi*, two *S. warneri* and four *S. lugdunensis*).

Methicillin resistance

Methicillin resistance was determined phenotypically by cefoxitin susceptibility. Detection of the *mecA* gene by PCR was utilized as a genotypic test for methicillin resistance. The PCR-amplified products were 310 bp in size. As shown in Table 1, *S. epidermidis* isolates had the highest rate of cefoxitin resistance (70%, 14/20), while *S. aureus* had the highest rate of detection of the *mecA* gene (45%, 9/20). Other CoNS isolates showed the lowest level of methicillin resistance. In *S. aureus*, all eight cefoxitin-resistant isolates were *mecA*-positive while in *S. epidermidis* isolates, the *mecA* test was positive in only half of the cefoxitin-resistant isolates. In other CoNS, the three cefoxitin-resistant isolates were also positive for the *mecA* gene. There was a statistically significant difference among different species in regard to cefoxitin susceptibility (*P*=0.003), but not for *mecA* gene detection (*P*=0.172).

Biofilm-forming ability

The biofilm-formation abilities of isolated *Staphylococcus* species were tested phenotypically using both TCP and CRA. Overall, as shown in Table 2, this ability was positive in rates of 63.8 and 57% by TCP and CRA methods, respectively. The isolated *Staphylococcus* species showed variation in this ability, as it was more apparent in *S. aureus* and other CoNS (70%, 66.7%, respectively by TCP) and (75%, 50%, respectively by CRA). Approximately half of the *S. epidermidis* isolates were biofilm formers. No significant statistical differences were detected among different species.

Relationship between methicillin resistance and biofilm-forming ability

To study this relationship, the biofilm-forming abilities of the tested *Staphylococcus* species (as measured by TCP) were distributed according to their methicillin-resistance pattern (measured phenotypically using cefoxitin susceptibility).

Here, TCP was used as it is the standard method for phenotypic detection of biofilm-forming ability [30]. As shown in Table 3, methicillin-sensitive species were either biofilm formers or non-formers with no statistically significant difference (*P*=0.25); while in the methicillin-resistant species, more than 70% (18/25) were biofilm formers with a statistically significant difference (*P*=0.05). All methicillin-resistant *S. aureus*, *S. haemolyticus* and *S. lugdunensis* were biofilm formers, while half of methicillin-resistant *S. epidermidis* isolates were biofilm formers.

Antibiotic susceptibility patterns of isolated strains

The susceptibility to vancomycin was assessed by determination of MIC values using the agar dilution method, while activities of other antibiotics were tested *in vitro* using the standardized CLSI disk diffusion method. The MIC values and inhibition zone sizes of tested quality control strains were within the normal ranges. No resistance was detected for vancomycin, as the MIC values were ≤0.5 µg ml⁻¹ for all 38 CoNS isolates, the MICs of the 15 *S. aureus* isolates were 1 µg ml⁻¹ and the remaining five *S. aureus* isolates had MICs of 2 µg ml⁻¹. Moreover, 100% of the tested isolates were sensitive to linezolid. The susceptibility patterns for the remainder of the tested antibiotics are presented in Table 4.

Resistance rates to other tested antibiotics varied from 30 to 60% for clindamycin and tetracycline, respectively, with *S. aureus* isolates, and from 30 to 80% for clindamycin and erythromycin, respectively, in *S. epidermidis* isolates. Overall, rifampicin was the most active antibiotic (after linezolid and vancomycin), as more than two-thirds of tested isolates (75.9%) were sensitive to it, while erythromycin was the least active (with a 65.5% resistance rate). Ciprofloxacin, clindamycin and cotrimoxazole were of the same overall sensitivity rate (63.8%). No significant statistical difference was detected in the activity of tested antibiotics among different tested *Staphylococcus* species, except for gentamicin (*P*=0.02).

Relationship of antibiotic susceptibility with methicillin resistance and biofilm-forming ability

Both vancomycin and linezolid had 100% sensitivity rates with all tested isolates, regardless of the methicillin

### Table 1. Pattern of methicillin resistance of isolated *Staphylococcus* species

<table>
<thead>
<tr>
<th><em>Staphylococcus</em> spp.</th>
<th>Cefoxitin</th>
<th>meca*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>6</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Other CoNS</td>
<td>15</td>
<td>83.3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>57</td>
<td>25</td>
</tr>
</tbody>
</table>

\( P \) value 0.003 0.172

*Detected using PCR and of 310 bp size.
resistance or biofilm-forming ability status of these isolates. The antibiotic susceptibility profiles obtained for the remainder of the tested antibiotics were distributed according to methicillin resistance (phenotypically assessed) and biofilm-forming ability (tested by TCP) to investigate the impact of these two virulence factors on the activities of the tested antibiotics (Table 5). It was noted that, for all tested antibiotics, most of the resistant isolates were sensitive to methicillin, as the lowest and highest sensitivity rates were 50 and 81 % of isolates resistant to rifampicin and clindamycin, respectively. The isolates sensitive to clindamycin and erythromycin showed considerable resistance to methicillin (56.8 and 55.0 %, respectively), while isolates sensitive to the other antibiotics showed high rates of methicillin sensitivity (51.4, 56.8, 61.5, 59.1 and 54.8 % for ciprofloxacin, cotrimoxazole, gentamicin, rifampicin and tetracycline, respectively). No significant statistical differences were found in the susceptibilities of tested antibiotics between methicillin-sensitive and -resistant species (all \( P > 0.05 \) in all cases), except for clindamycin (\( P = 0.005 \)). As regards biofilm-forming ability, most antibiotic-resistant isolates had a high forming ability (71.4, 76.2, 66.7, 57.9, 62.5, 57.1 and 74.1 % for ciprofloxacin, clindamycin, cotrimoxazole, erythromycin, gentamicin, rifampicin and tetracycline, respectively), and the sensitive isolates were found to have biofilm-forming ability rates ranging from 54.8 to 76.2 %. The in vitro activities of tested antibiotics showed no significant statistical differences according to biofilm-forming ability (all \( P > 0.05 \)). It is apparent from these results that the susceptibility profiles for the tested antibiotics could not be affected by the methicillin resistance or biofilm-forming ability of tested \textit{Staphylococcus} species.

**DISCUSSION**

The current study aimed to identify \textit{Staphylococcus} species causing CRBSIs in our tertiary hospital and to study their virulence as regards methicillin resistance, biofilm formation and antibiotic resistance, as well in vitro activities of vancomycin and linezolid against them.

Both \textit{S. aureus} and \textit{S. epidermidis} were isolated at equally higher rates (20 isolates; 34.5 % each) than the other CoNS species: nine (15.5 %) \textit{S. haemolyticus}, three (5.2 %) \textit{S. schleiferi}, two (3.4 %) \textit{S. warneri} and four (6.9 %) isolates of \textit{S. lugdunensis}. Our findings are in accordance with other studies in the literature \[10, 33\] which found that \textit{S. aureus} and

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**Table 2. Distribution of tested \textit{Staphylococcus} species according to biofilm-forming ability**

<table>
<thead>
<tr>
<th>\textit{Staphylococcus} spp.</th>
<th>TCP</th>
<th>CRA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>\textit{S. aureus}</td>
<td>14</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>\textit{S. epidermidis}</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Other CoNS</td>
<td>12</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.586</td>
<td>0.124</td>
<td></td>
</tr>
</tbody>
</table>

TCP, tissue culture plate; CRA, Congo red agar; \( n \), number.

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**Table 3. Distribution of biofilm-forming ability according to methicillin-susceptibility pattern**

<table>
<thead>
<tr>
<th>Methicillin susceptibility</th>
<th>TCP</th>
<th>Species (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( +ve )</td>
<td>\textit{S. aureus}</td>
</tr>
<tr>
<td>Sensitive</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>–ve</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Resistant†</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>–ve</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

TCP, tissue culture plate; \( n \), number.

*No significant statistical difference.
†Significant statistical difference.
S. epidermidis were the commonest causes of CRBSIs and infections related to implanted prosthetic materials. Moreover, coagulase-negative Staphylococcus species (including S. epidermidis) were found to play key roles in CRBSIs, as proved by the studies in [34, 35] (29.6 and 31%, respectively), and this could be explained by the origin of CoNS as they are part of skin flora and infection can occur readily due to contamination of IV catheters.

Methicillin resistance was tested phenotypically and genotypically. The overall resistance rates detected phenotypically and genotypically were 43 and 32.7%, respectively. This relatively high rate of methicillin resistance in different Staphylococcus species is consistent with many previous studies [35–38], demonstrating that MRSA and MRCoNS still cause a considerable number of hospital-acquired catheter-related infections, and the mecA gene is the main

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
<th>S. haemolyticus</th>
<th>S. lugdunensis</th>
<th>S. warneri</th>
<th>S. schleiferi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>12 (60.0%)</td>
<td>13 (65.0%)</td>
<td>8 (88.9%)</td>
<td>1 (25.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>8 (40.0%)</td>
<td>7 (35.0%)</td>
<td>1 (11.1%)</td>
<td>3 (75.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>S</td>
<td>14 (70.0%)</td>
<td>14 (70.0%)</td>
<td>6 (66.7%)</td>
<td>2 (50.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>6 (30.0%)</td>
<td>6 (30.0%)</td>
<td>3 (33.3%)</td>
<td>2 (50.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>S</td>
<td>11 (55.0%)</td>
<td>13 (65.0%)</td>
<td>8 (88.9%)</td>
<td>1 (25.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>9 (45.0%)</td>
<td>7 (35.0%)</td>
<td>1 (11.1%)</td>
<td>3 (75.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>9 (45.0%)</td>
<td>4 (20.0%)</td>
<td>4 (44.4%)</td>
<td>0 (0.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>11 (55.0%)</td>
<td>16 (80.0%)</td>
<td>5 (55.6%)</td>
<td>4 (100.0%)</td>
<td>0 (0.0%)</td>
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<tr>
<td>Gentamicin</td>
<td>S</td>
<td>9 (45.0%)</td>
<td>5 (25.0%)</td>
<td>8 (88.9%)</td>
<td>1 (25.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>11 (55.0%)</td>
<td>15 (75.0%)</td>
<td>1 (11.1%)</td>
<td>3 (75.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>S</td>
<td>15 (75.0%)</td>
<td>11 (55.0%)</td>
<td>9 (100.0%)</td>
<td>4 (100.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5 (25.0%)</td>
<td>9 (45.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>8 (40.0%)</td>
<td>11 (55.0%)</td>
<td>8 (88.9%)</td>
<td>1 (25.0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12 (60.0%)</td>
<td>9 (45.0%)</td>
<td>1 (11.1%)</td>
<td>3 (75.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

n, number; S, sensitive; R, resistant.

*No significant statistical difference.
†Significant statistical difference.

Table 4. Antibiotic susceptibility patterns of tested Staphylococcus species as detected using the disk diffusion method

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Methicillin sensitivity*</th>
<th>TCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (%)</td>
<td>Resistant (%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>51.4</td>
<td>48.6</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>66.7</td>
<td>33.3</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>43.2</td>
<td>56.8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>81.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>56.8</td>
<td>43.2</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>57.1</td>
<td>42.9</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>45.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

*Assessed phenotypically by cefoxitin susceptibility.

Table 5. Distribution of antibiotic susceptibility profiles according to methicillin resistance and biofilm-forming ability

All values are expressed in %. TCP, tissue culture plate. No significant statistical differences were found in susceptibilities of tested antibiotics according to either methicillin sensitivity or biofilm-forming ability, except for clindamycin susceptibility with methicillin sensitivity (P=0.005).
underlying cause for methicillin resistance. In our study, the mecA gene was found in nine isolates of *S. aureus* while only eight of these were phenotypically resistant; this means that genetic resistance was not expressed in one isolate, and this could be explained by the other factors required for mecA gene expression, either internal factors such as Fem or external factors such as pH, salt concentration, osmolarity and medium composition [17, 39]. On the other hand, in *S. epidermidis*, only half of the phenotypically resistant isolates were positive for the mecA gene and this may be explained by the association of this species to other resistance mechanisms such as hyper-production of beta-lactamases or production of modified normal PBPs, which have low affinity for methicillin [40].

In regard to the biofilm-forming ability of the isolated *Staphylococcus* species, we assessed this using CRA and TCP methods. The overall results of both methods revealed that around 60% of isolated *Staphylococcus* species were biofilm formers. Moreover, both methods showed that more than two-thirds of *S. aureus* and other CoNS were biofilm formers, while this ability was around 50% in *S. epidermidis*. Our results agree with those of Singhai *et al.* [41]. Biofilm formation is a crucial virulence factor in CRBSIs, as it enables the causative bacteria to establish persistent bloodstream infection in addition to marked resistance to antimicrobials [42, 43]. Investigating the relationship between biofilm formation capacity and methicillin resistance in our isolates revealed a significant increase in biofilm-forming ability among MRSA and MRCoNs. This finding was more prominent in methicillin-resistant *S. aureus*, *S. haemolyticus* and *S. lugdunensis*, as all were biofilm formers, while half of the methicillin-resistant *S. epidermidis* (MRSE) isolates were biofilm formers. The presence of both of these factors renders the causative agent more virulent.

Antibiotic susceptibility testing of tested isolates showed full sensitivity (100%) of all isolates to vancomycin and linezolid. The positive result for methicillin resistance did not affect this sensitivity pattern, as both antibiotics were active against MRSA and all MRCoNs, and these results coincide with those of Khanna *et al.* [44] and Parameswaran *et al.* [10], who found 100% sensitivity to vancomycin and linezolid among all *Staphylococcus* species isolated from CRBSIs. The difference in susceptibility of vancomycin and linezolid compared to methicillin can be explained by the occurrence of resistance to these two drugs by mechanisms other than the mecA gene, including, for example, *cfr* gene and G2576T mutation in the 23S rRNA for linezolid [45] and rpoB mutation for vancomycin [46].

Moreover, the sensitivities to these two drugs were not affected by the positivity of our tested isolates for biofilm formation. This may be explained by the fact that our susceptibility testing was carried out on the isolates in a planktonic state and not in biofilm form. If this antibiotic activity testing was performed on in vitro *Staphylococcus* biofilms, it could give different results with a tendency towards resistance, as found by Mataraci and Dosler [47] and Oliveira *et al.*, who found an increase in MICs for many antibiotics, including vancomycin, against biofilm cells as compared to planktonic cells of *S. aureus* and CoNS species [48].

Overall, the results of this study showed high and variable degrees of multi-resistance to tested antibiotics (varying according to species tested), with rifampicin being the most active and erythromycin the least. No significant statistical differences were found in resistance rates among the tested isolates according to their methicillin-resistance status, which may be explained as mentioned above by the presence of different mechanisms for antibiotic resistance. Although no significant differences were detected in the resistance patterns for biofilm-formation ability, it was found that most of the resistant isolates were biofilm formers, which is in line with a study by Singhai *et al.* [41]. The presence of such methicillin-resistant, biofilm-forming *Staphylococcus* species in a hospital environment is a major risk factor for the development of more aggressive and challenging nosocomial infections, including CRBSIs that are usually resistant to treatment, and this reinforces the importance of the proper use and insertion of venous catheters to avoid the occurrence of CRBSIs.

In conclusion, phenotypic methicillin resistance was more prevalent in *S. epidermidis* than in *S. aureus* and other CoNS; the mecA gene was the major underlying cause of methicillin resistance in MRSA and other methicillin-resistant CoNS. Most of the *Staphylococcus* species were biofilm producers, and MRSA and the other methicillin-resistant CoNS showed a better biofilm-formation ability than MRSE isolates. Vancomycin and linezolid exerted the highest *in vitro* activity against all *Staphylococcus* species, and such activity was not affected by methicillin resistance or biofilm forming ability. Rifampicin was the most active among the other tested antibiotics. Consequently, vancomycin, linezolid and rifampicin could provide antibiotics active against MRSA, MRSE and other MRCoNs isolated from cases of CRBSIs. More studies including different *staphylococci* species from CRBSIs are needed to obtain more detail on the virulence factors and activities of vancomycin, linezolid and other antibiotics against *in vitro* biofilms formed by MRSA, MRSE and other MRCoNS.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
The study was approved by the research ethical committee of the Faculty of Medicine, Suez Canal University, and informed written consent was obtained from each patient included in this study.
References


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