**INTRODUCTION**

*Staphylococcus aureus* (*S. aureus*) is a well-known human pathogen that causes a variety of community and healthcare-associated infections (HAIs); including, toxic shock syndrome, infective endocarditis, skin and soft tissue infections, septic arthritis, pneumonia, meningitis, and bacteremia. Before the dawn of antimicrobials, *S. aureus* was implicated in 80% of human deaths; however, with the introduction of the earliest antibiotics, including penicillin, a considerable reduction in human mortality had occurred.

The first cases of methicillin-resistant *S. aureus* (MRSA) were recorded in the early 1960s. Due to two separate mechanisms, the breakdown of the antibiotics' β-lactam ring by β-lactamas and the development of a low-affinity penicillin-binding protein (PBP2a), which is encoded by the *meC* gene, MRSA are resistant to β-lactams and β-lactamase inhibitor combinations. Vancomycin was the first choice for treatment of MRSA infections for a long time, however, emergence of resistant strains has been increased recently.

Linezolid is the first generation of oxazolidinone antibiotics that inhibits bacterial protein biosynthesis. It was approved in 2000, for the treatment of infections caused by Gram-positive cocci including MRSA and vancomycin resistant strains. Despite the therapeutic efficacy of this drug, evolution of linezolid resistance amongst *S. aureus*, including MRSA, have been described worldwide. The fundamental mechanisms of resistance are point mutations in the 23S rRNA V domain, amino acid alterations in the ribosomal proteins L3, L4, and L22, and the acquisition of *cfr* resistant gene, which is linked to numerous resistance phenotypes.

In 2014, the next-generation oxazolidinone, tedizolid (formerly torezolid), was authorized for the treatment of acute bacterial skin and skin structure infections. Similar to linezolid, it interferes with bacterial protein biosynthesis. However, it has a broader antibacterial spectrum with less adverse effects compared to linezolid. In vitro, it demonstrates a potent activity against several vancomycin-resistant Gram-positive organisms including *Staphylococci, Enterococci,* and *Streptococci*. Additionally, it works against isolates of linezolid-resistant *S. aureus* that harbor the *cfr* gene.

Given the rising incidence of antibiotic-resistant *S. aureus* is associated with limited antimicrobial arsenal.
thus, it is crucial to evaluate the efficacy of the novel antimicrobials against these superbugs. Thereby, this study intended to (i) determine the in vitro susceptibility of various \textit{S. aureus} clinical isolates, from Mansoura University Hospitals (MUHs), to linezolid and tedizolid, and (ii) identify the underlying molecular mechanisms.

### METHODOLOGY

**Ethical approval statement:**

The Declaration of Helsinki's recommendations were followed in the execution of the current investigation. The ethical committee, Faculty of Medicine, Mansoura University, Egypt gave its approval to the study protocol (R.19.04.491). Informed written consents were provided from all participants.

**Study design and samples collection:**

In this prospective study, different clinical samples including wound swabs, blood, urine, and respiratory samples were taken from patients who had been admitted to different departments of MUHs, Egypt, and had clinical signs suggestive of HAIs between January 2021 and March 2022.

**Processing of clinical samples and bacterial identification:**

Samples' processing and bacterial identification were undertaken in the bacteriology laboratory, Microbiology Diagnostics and Infection Control Unit (MDICU), Mansoura Faculty of Medicine, Egypt, according to the standard microbiological methods. All culture media were purchased from Oxoïd Ltd., UK. \textit{S. aureus} isolates were identified based on colony shape, Gram staining characters, biochemical reactions as catalase, coagulase, and DNAse tests, as well as the development of yellow colonies on mannitol salt agar plates (Oxoïd Ltd., UK).

**Antibiotic sensitivity testing:**

Susceptibility testing was conducted using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotic discs purchased from Oxoïd Ltd. UK were utilized. Ampicillin (10 \(\mu\)g), cefoxime (30 \(\mu\)g), ampicillin/sulbactam (10/10 \(\mu\)g), erythromycin (15 \(\mu\)g), gentamicin (10 \(\mu\)g), amikacin (30 \(\mu\)g), trimethoprim/sulfamethoxazole (25 \(\mu\)g), imipenem (10 \(\mu\)g), levofloxacin (5 \(\mu\)g), and vancomycin (30 \(\mu\)g). Linezolid (30 \(\mu\)g) and tedizolid (20 \(\mu\)g) discs were obtained from Liofilchem Inc., Italy. The \textit{S. aureus} ATCC 25923 (Naval Medical Research Unit Three, Cairo, Egypt; NAMRU-3) was used as a quality control strain in each batch of antibiotic testing.

**Screening for MRSA isolates:**

The cefoxitin disc diffusion method (30 \(\mu\)g, Oxoïd Ltd., UK) was used to screen for MRSA isolates according to the CLSI guidelines. Inhibition zones \(\leq 21\) mm around the cefoxitin disc was identified as MRSA, while isolates with inhibition zones \(\geq 22\) mm were defined as methicillin-susceptible \textit{S. aureus} (MSSA).

**Evaluation of the minimum inhibitory concentrations (MICs) of linezolid and tedizolid:**

The MICs of linezolid and tedizolid against the \textit{S. aureus} isolates were evaluated using the broth microdilution method. Linezolid and tedizolid MICs were interpreted following the CLSI recommendations, and the updated breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Resistance was determined if the MIC was \(> 4\) \(\mu\)g/L for linezolid, and \(> 0.5\) \(\mu\)g/L for tedizolid. \textit{S. aureus} ATCC 29213 was employed as the reference strain.

**Genomic DNA extraction:**

Overnight \textit{S. aureus} cultures were used to extract DNA using the boiling procedure, which involved suspending pure \textit{S. aureus} colonies in 100\(\mu\)l of sterile phosphate buffered saline, then 10 minutes of boiling at 100\(^\circ\)C, and left to cool. Bacterial suspensions were kept at \(-80\)\(^\circ\)C until subsequent amplification using polymerase chain reaction (PCR).

**Molecular Detection of \textit{mecA} and \textit{cfr} genes:**

All used primers were obtained from Sigma, Aldrich. The PCR conditions for \textit{mecA} gene (determinant of MRSA), and \textit{cfr} gene (determinant of linezolid resistance) are listed in table 1.

After amplification, in each PCR reaction 5\(\mu\)l of the amplicons were removed and put through 1.5% agarose gel electrophoresis to determine the sizes of the amplification products by comparing them to a molecular marker of 1000 bp (Lonza Rockland, Inc., USA).

**Table 1: Oligonucleotides sequences and sizes of different primers used in the amplification of \textit{mecA} and \textit{cfr} genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5'→3')</th>
<th>Amplicon size (bp)</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>F-AAAAATCGATGTTAAAGGTGGC&lt;br&gt;R-AGTTCTGCGATACGGGATTTCGC</td>
<td>532</td>
<td>Denaturation at 94°C for 5 min, 40 cycles (30 sec at 94°C, 30 sec at 55°C, 60 sec at 72°C), final extension at 72°C for 5 min.</td>
</tr>
<tr>
<td>cfr</td>
<td>F- TGA AGT ATA AAG CAG GTT GGG AGT CA&lt;br&gt;R- ACC ATA TAA TTG ACC ACA AGC AGC</td>
<td>746</td>
<td>Denaturation at 94°C for 2 min, 30 cycles (10 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C), final extension of 7 min at 72°C.</td>
</tr>
</tbody>
</table>

Note: bp, base pair.
Statistical Analysis

All data were graphed and statistically evaluated using the SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Numbers and percentages were used to describe descriptive data. The relationship between categorical variables was evaluated using the Pearson's Chi-squared ($\chi^2$) test. The association between various risk factors and linezolid resistance was investigated using univariate logistic regression analysis. Statistical significance was defined as $P$-values $<$ 0.05.

RESULTS

Bacterial isolates and patients’ characteristics:

A total of 113 consecutive, non-duplicate $S$. aureus isolates were found during the study period in various clinical samples, primarily from wound swabs (53/113, 46.9%), followed by blood (28/113, 24.8%), urine (19/113, 16.8%), and respiratory samples (13/113, 11.5%). Amongst the study cohort, 64 (56.6%) were males, while 49 (43.4%) were females. The infected patients' age was $51 \pm 12.7$ years (range; 19–70 years).

Antibiotic sensitivity pattern of the test isolates by disc diffusion method:

In vitro antibiotic susceptibility testing revealed high resistance rate among the investigated $S$. aureus isolates to ampicillin (100%), whereas 108 (95.6%) of the isolates were resistant to each of cefuroxime and trimethoprim/sulfamethoxazole, gentamicin 95 (84.1%), ampicillin/sulbactam 91 (80.5%), amikacin 90 (79.6%), erythromycin 86 (76.1%), levofloxacin 83 (73.5%), and imipenem 56 (49.6%). Methicillin resistance was identified in 19 (16.8%) of the test isolates. On the other hand, resistance to vancomycin was identified in 17 (15.0%) of the isolates. Linezolid resistance was observed in 11 (9.7%) of the isolates, where all of them were methicillin-resistant. Of the linezolid-resistant MRSA isolates, 72.7% were recovered from wound swabs, whereas 27.3% were from blood samples. Notably, tedizolid retained significant activity against all of our $S$. aureus isolates, regardless of methicillin susceptibility (figure 1).

![Fig. 1: Resistance profile of Staphylococcus aureus clinical isolates.](image)

The MICs of linezolid and tedizolid by broth microdilution method:

Tedizolid maintained greater efficacy compared to linezolid against all $S$. aureus isolates regardless of methicillin susceptibility. The MIC$_{50}$ and MIC$_{90}$ of tedizolid for both MSSA and MRSA were 0.25 µg/L and 0.5 µg/L, respectively. On the other side, the MIC$_{50}$ and MIC$_{90}$ of linezolid against MSSA were 0.5 and 1 µg/L, respectively, and 2 µg/L for MRSA. The MICs of tedizolid ranged from 0.12 to 0.5 µg/L, lower than those of linezolid for both MSSA and MRSA, whilst, the MICs of linezolid ranged from 0.5 to 2 µg/L for MSSA and from 0.5 to $>4$ µg/L for MRSA.

All of the MRSA isolates (n= 11) were resistant to linezolid with MIC $>$ 4 µg/L (table 2, figure 2).
Table 2: The minimum inhibitory concentrations (MICs) of linezolid and tedizolid against *S. aureus* isolates

<table>
<thead>
<tr>
<th>Bacterial strain (n= 113)</th>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>Resistant strains No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>MSSA (n= 94)</td>
<td>Linezolid</td>
<td>0.5–2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Tedizolid</td>
<td>0.12–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>MRSA (n= 19)</td>
<td>Linezolid</td>
<td>0.5–&gt;4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Tedizolid</td>
<td>0.12–0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Abbreviations: MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

Fig. 2: Cumulative distribution of the minimum inhibitory concentrations (µg/mL) of linezolid and tedizolid against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

Risk factors associated with linezolid resistance:
In the univariate analysis, previous surgery (OR= 3.67, 95% CI: 1.40–9.62; *P* = 0.002), previous hospitalization (OR= 0.21, 95% CI: 0.08–0.58; *P*= 0.002), and ICU admission (OR= 0.07, 95% CI: 0.01–0.68; *P*= 0.013) were associated with acquisition of linezolid resistance (table 3).
Table 3: Risk factors for and outcome of linezolid resistance amongst the identified MRSA isolates

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Linezolid susceptible MRSA (n= 8)</th>
<th>Linezolid resistant MRSA (n= 11)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (± SD)</td>
<td>39.6 ± 15.7 (21–61)</td>
<td>44.5 ± 10.3 (29–56)</td>
<td></td>
<td>0.287</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (62.5)</td>
<td>10 (90.9)</td>
<td>0.17 (0.01–2.0)</td>
<td>0.134</td>
</tr>
<tr>
<td>Female</td>
<td>3 (37.5)</td>
<td>1 (9.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comorbidity</td>
<td>6 (75.0)</td>
<td>5 (45.5)</td>
<td>3.6 (0.49–26.4)</td>
<td>0.198</td>
</tr>
<tr>
<td>Previous surgery</td>
<td>0 (0.0)</td>
<td>8 (72.7)</td>
<td>3.67 (1.40–9.62)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Previous hospitalization</td>
<td>3 (37.5)</td>
<td>11 (100.0)</td>
<td>0.21 (0.08–0.58)</td>
<td>0.002*</td>
</tr>
<tr>
<td>ICU admission</td>
<td>2 (25.0)</td>
<td>9 (81.8)</td>
<td>0.07 (0.01–0.68)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Indwelling devices</td>
<td>1 (12.5)</td>
<td>5 (45.5)</td>
<td>0.17 (0.02–1.90)</td>
<td>0.127</td>
</tr>
<tr>
<td>Prior Linezolid use</td>
<td>2 (25.0)</td>
<td>5 (45.5)</td>
<td>0.40 (0.06–2.93)</td>
<td>0.361</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>0 (0.0)</td>
<td>7 (63.6)</td>
<td>3 (1.35–6.68)</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval; ICU, intensive care unit. *P < 0.05 (statistically significant).

Distribution of mecA and cfr genes:
All of the identified MRSA isolates by phenotypic methods (n= 19) were found to harbor the mecA gene. The cfr gene was detected in 57.9% of linezolid-resistant MRSA isolates (11/19), whereas none of MSSA isolates carried this gene, with a statistically significant difference between both strains (P= 0.001).

DISCUSSION

In recent decades, the unwise use of antibiotics culminated into spread of resistant S. aureus isolates, especially MRSA. Worryingly, isolates with diminished susceptibility to vancomycin have been described worldwide and are associated with unfavorable prognosis. Though both linezolid and tedizolid were approved for management of S. aureus infections worldwide, no much data have been published from Egypt in this context.

This study recovered 113 S. aureus isolates from the enrolled patients. The bulk of the isolates were obtained from wound swabs (46.9%), followed by blood (24.8%), that is consistent with earlier research, according to which S. aureus was the main pathogen found in blood stream infections, skin and soft tissue infections, and post-operative wound infections. In line with earlier data from Egypt, the antibiotic sensitivity pattern of S. aureus isolates revealed a significant prevalence of multidrug resistance.

Increased rates of vancomycin-resistant S. aureus (VRSA) is an alarm for health institutions worldwide, as it is considered the main therapy for MRSA infections. Therefore, decreased susceptibility to vancomycin amongst S. aureus strains is a matter of concern. Noteworthy, about 15% of our isolates were resistant to vancomycin, in keeping with the findings of ElSayed et al., where 13.8% resistance rate to vancomycin was identified.

In our study, 16.8% of the test isolates were methicillin-resistant, close to an earlier Egyptian study, where MRSA constituted 15% of the isolates. On the flip side, other studies from Egypt reported higher rates of MRSA from infected patients (43.8%, and 76.6%), respectively. In addition, another study from Japan showed increased infection rates by MRSA. A previous study by Montazeri et al., involving burn patients, reported that 88.6% of the analyzed isolates were MRSA. Inadequate infection prevention and control measures associated with indiscriminate consumption of antibiotics are among the major causes of acquisition and propagation of these superbugs, which restrains the currently available treatment options for MRSA infections.

In the current study, S. aureus isolates demonstrated high susceptibility patterns to both linezolid and tedizolid. All of the test isolates were susceptible to tedizolid regardless of being MSSA or MRSA, while 90.3% of the isolates were sensitive to linezolid. Additionally, the MICs of tedizolid were inferior to that of linezolid as summarized in table 2 and figure 2. Our findings are compatible with other published reports, where tedizolid showed superior activity compared to linezolid and other antimicrobials. In a previous work by Bensaci and Sahm, a high activity of tedizolid against S. aureus isolates was identified compared to linezolid; however, tedizolid resistance was detected in 0.2% of S. aureus isolates. This raises questions about the possible mechanisms of tedizolid resistance, and increase the need to revise strategies undertaken in healthcare setting to confront unlimited emergence of antimicrobial resistance. In the existing
study, all of the isolates were sensitive to tedizolid, while 9.7% were resistant to linezolid. An earlier study conducted at our institution observed 9.1% resistance to linezolid, which endorses our results. On the other hand, a previous study conducted in Korea, described high linezolid resistance amongst S. aureus isolates (14.8%). This discrepancy may be due to variation in antimicrobial policies. The current study showed that tedizolid had high activity against linezolid-resistant MRSA isolates that carry the cfr gene, consistent with other investigations that documented potent effect of tedizolid against cfr-positive, linezolid-resistant S. aureus isolates.

The present study noted that undergoing surgery, previous hospitalization, and ICU admission for long duration were significantly correlated with linezolid resistance as illustrated in table 3. Recently issued articles publicized that patients’ admission to hospitals, and ICU for prolonged duration was significantly associated with acquisition of linezolid resistance. This may be due to occurrence of cross-resistance between virulent strains, and deficient immunity among patients in ICU, along with increased use of linezolid in prophylactic regimen against HAIs, particularly surgical infections.

In an attempt to uncover the underlying molecular mechanisms of linezolid resistance amongst our S. aureus isolates, PCR was performed to check for the existence of the cfr gene. Our analyses proved the existence of cfr gene in all linezolid-resistant isolates, congruent with a previous study from France. Also, these findings support results from previous studies conducted in Egypt.

CONCLUSIONS

Linezolid has potent in vitro antibacterial activity against S. aureus isolates, but emergence of resistant strains is catastrophic. Multidrug-resistant S. aureus including vancomycin and linezolid-resistant strains, exhibit high susceptibility rates to tedizolid. Future studies are recommended to evaluate the activity of tedizolid against various Gram-positive pathogens causing HAIs, and to investigate the possible mechanisms of resistance.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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