ORIGINAL ARTICLE

Tedizolid versus linezolid activity against *Staphylococcus aureus* clinical isolates

[1]Rasha M. Elnagar[*], [2]Rasha Mahmoud, Rania A. El-Kady[1,3]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Internal Medicine, Nephrology and Dialysis Unit, Mansoura University, Mansoura, Egypt
[3]Department of Pathological Sciences, Fakeeh College for Medical Sciences, Jeddah, Kingdom of Saudi Arabia

Key words:
Linezolid resistance; Staphylococcus aureus; cfr gene; tedizolid; minimum inhibitory concentration (MIC)

Corresponding Author:
Rasha Mokhtar Elnagar
Department of Medical Microbiology & Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Tel.: +201095129425
drrasha_m@mans.edu.eg

ABSTRACT

Background: *In recent years, emergence of linezolid-resistant strains has gained a considerable clinical concern. Tedizolid, a relatively new antibiotic, showed strong effectiveness against S. aureus; however, few data are available from Egypt in this regard.* ***Objectives:*** *This study aimed to assess the in vitro susceptibility of S. aureus clinical isolates from Mansoura University Hospitals (MUHs), Egypt to linezolid and tedizolid, and to unveil the underlying molecular mechanisms for resistance.* ***Methodology:*** *In vitro susceptibility of 113 S. aureus isolates from various clinical samples was determined by disc diffusion method. The broth microdilution method was used to determine the MICs of linezolid and tedizolid. Detection of mecA and cfr genes among MRSA and linezolid-resistant isolates, respectively was performed using polymerase chain reaction (PCR).* ***Results:*** *In this study, 16.8% of S. aureus isolates were methicillin-resistant. On the other hand, resistance to vancomycin and linezolid was identified in 15%, and 9.7 % of the isolates, respectively. All the isolates were susceptible to tedizolid. In comparison between methicillin-sensitive S. aureus (MSSA) and MRSA as regards tedizolid and linezolid susceptibility, tedizolid retained low MIC_{50}, and MIC_{90} (0.25, and 0.5 µg/L) for all S. aureus isolates. On the other hand, the MICs of linezolid were 0.5–2 µg/L for MSSA, and 0.5–>4 µg/L for MRSA isolates. All MRSA isolates (n= 19) were found to harbor the mecA gene. The cfr gene was identified in 57.9% of linezolid-resistant MRSA isolates.* ***Conclusion:*** *Tedizolid is highly effective against S. aureus clinical isolates, including linezolid-resistant strains.*

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[1]. 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[2].

The first cases of methicillin-resistant *S. aureus* (MRSA) were recorded in the early 1960s[3]. Due to two separate mechanisms, the breakdown of the antibiotics' β-lactam ring by β-lactamases and the development of a low-affinity penicillin-binding protein (PBP2a), which is encoded by the *mecA* gene, MRSA are resistant to β-lactams and β-lactamase inhibitor combinations[4]. Vancomycin was the first choice for treatment of MRSA infections for a long time, however, emergence of resistant strains has been increased recently[5].

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[6]. Despite the therapeutic efficacy of this drug, evolution of linezolid resistance amongst *S. aureus*, including MRSA, have been described worldwide[7]. 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[8].

In 2014, the next-generation oxazolidinone, tedizolid (formerly torezolid), was authorized for the treatment of acute bacterial skin and skin structure infections[9]. Similar to linezolid, it interferes with bacterial protein biosynthesis. However, it has a broader antibacterial spectrum with less adverse effects compared to linezolid[10]. 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[11].

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 *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 Oxoid Ltd., UK. *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 (Oxoid Ltd., UK)[12, 13].

Antibiotic sensitivity testing:

Susceptibility testing was conducted using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[14]. The following antibiotic discs purchased from Oxoid Ltd. UK were utilized. Ampicillin (10 µg), cefuroxime (30 µg), ampicillin/sulbactam (10/10 µg), erythromycin (15 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (25 µg), imipenem (10 µg), levofloxacin (5 µg), and vancomycin (30 µg). Linezolid (30 µg) and tedizolid (20 µg) discs were obtained from Liofilchem Inc., Italy. The *S. aureus* ATCC 25923 (Naval Medical Research Unit Three, Cairo, Egypt; NAMRU-3) was used a quality control strain in each batch of antibiotic testing.

Screening for MRSA isolates:

The cefoxitin disc diffusion method (30 µg, Oxoid Ltd., UK) was used to screen for MRSA isolates according to the CLSI guidelines[14]. Inhibition zones ≤ 21 mm around the cefoxitin disc was identified as MRSA, while isolates with inhibition zones ≥ 22 mm were defined as methicillin-susceptible *S. aureus* (MSSA).

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

The MICs of linezolid and tedizolid against the *S. aureus isolates* were evaluated using the broth microdilution method. Linezolid and tedizolid MICs were interpreted following the CLSI recommendations[15], and the updated breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[16]. Resistance was determined if the MIC was > 4 µg/L for linezolid, and > 0.5 µg/L for tedizolid. *S. aureus* ATCC 29213 was employed as the reference strain.

Genomic DNA extraction:

Overnight *S. aureus* cultures were used to extract DNA using the boiling procedure, which involved suspending pure *S. aureus* colonies in 100µl of sterile phosphate buffered saline, then 10 minutes of boiling at 100°C, and left to cool[17]. Bacterial suspensions were kept at −80°C until subsequent amplification using polymerase chain reaction (PCR).

Molecular Detection of *mecA* and *cfr* genes:

All used primers were obtained from Sigma, Aldrich. The PCR conditions for *mecA* gene (determinant of MRSA), and *cfr* gene (determinant of linezolid resistance) are listed in table 1[18, 19].

After amplification, in each PCR reaction 5µl 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 *mec*A and *cfr* genes

Gene	Primer sequence (5′→3′)	Amplicon size (bp)	PCR conditions
mecA	F-AAAATCGATGGTAAAGGTTGGC R-AGTTCTGCAGTACCGGATTTGC	532	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.
cfr	F- TGA AGT ATA AAG CAG GTT GGG AGT CA R- ACC ATA TAA TTG ACC ACA AGC AGC	746	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.

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 ± 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).

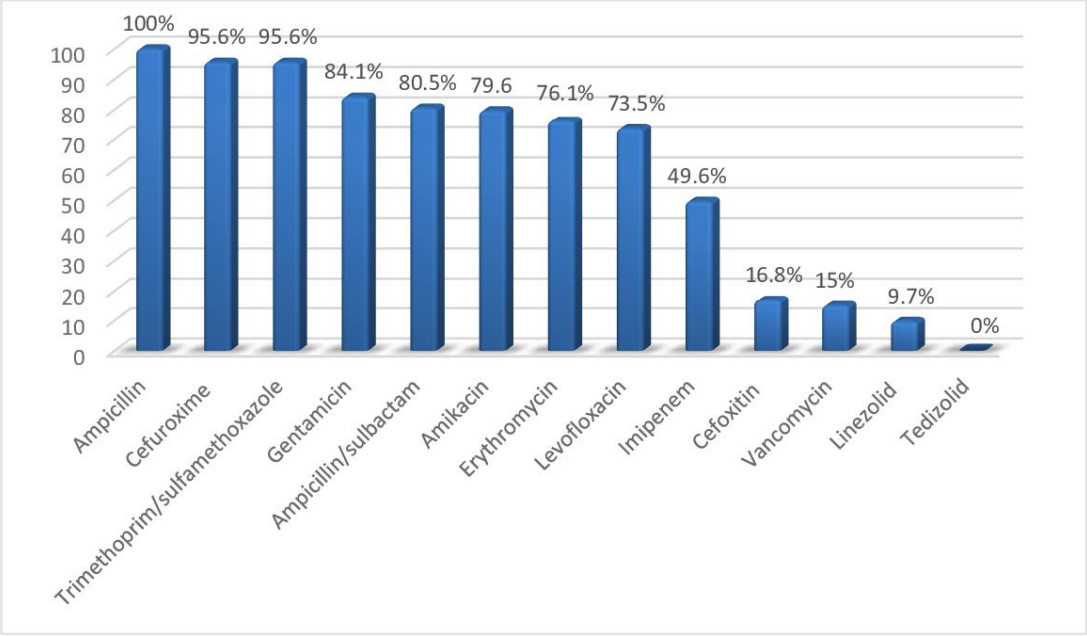

Fig. 1: Resistance profile of *Staphylococcus aureus* clinical isolates.

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 0.5µg/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*

Bacterial strain (n= 113)	Drug	MIC (µg/ml)			Resistant strains No.
		Range	MIC_{50}	MIC_{90}	
MSSA (n= 94)	Linezolid	0.5–2	0.5	1	0
	Tedizolid	0.12–0.5	0.25	0.5	0
MRSA (n= 19)	Linezolid	0.5–>4	2	2	11
	Tedizolid	0.12–0.5	0.25	0.5	0

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

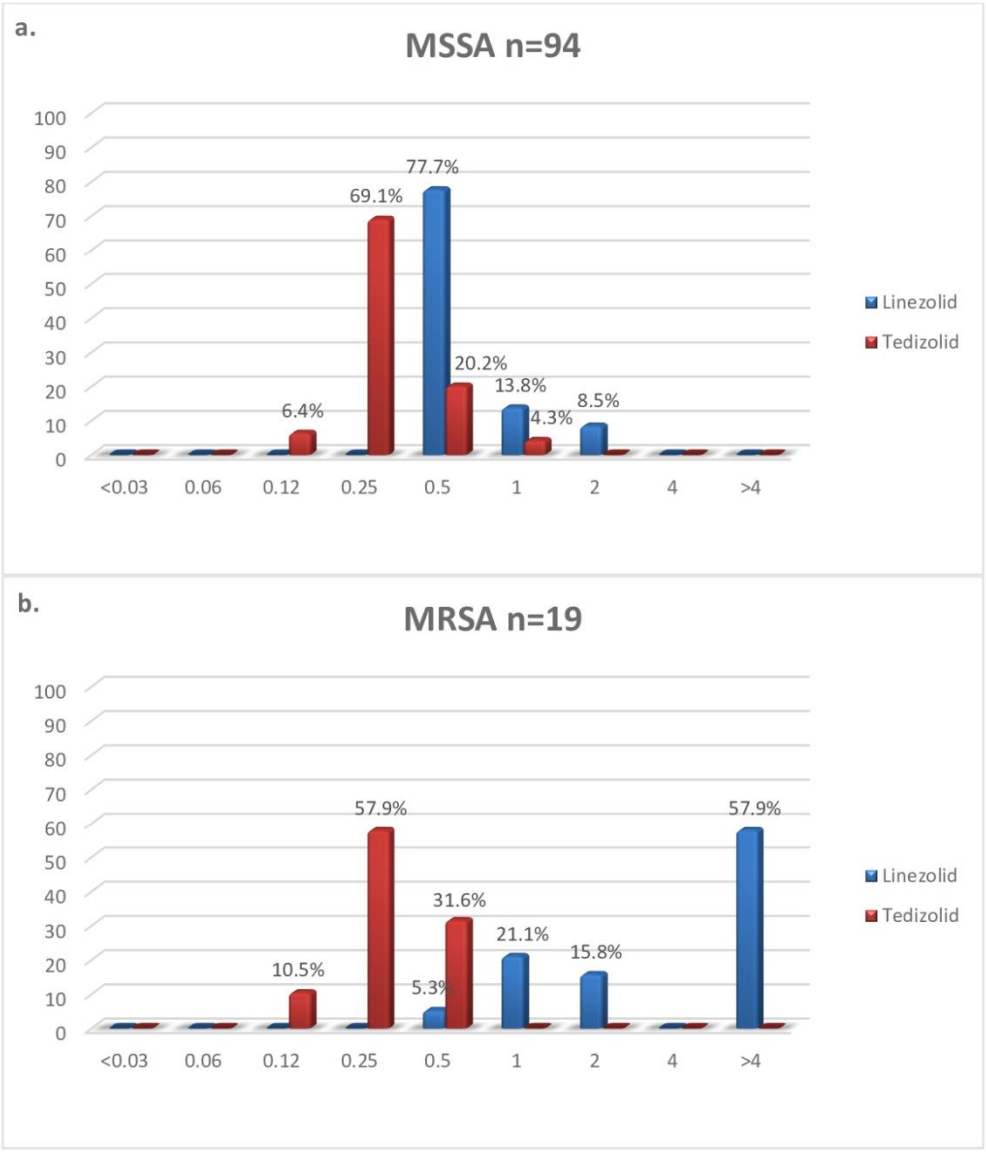

Fig. 2: Cumulative distribution of the minimum inhibitory concentrations (µg/mL) of linezolid and tedizolid against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

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

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

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[20]. 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[21]. In line with earlier data from Egypt, the antibiotic sensitivity pattern of *S. aureus* isolates revealed a significant prevalence of multidrug resistance[22].

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[23]. 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[24].

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[25]. On the flip side, other studies from Egypt reported higher rates of MRSA from infected patients (43.8%, and 76.6 %), respectively[24, 26]. In addition, another study from Japan showed increased infection rates by MRSA[27]. A previous study by Montazeri et al., [28] 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[29].

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[30, 31]. Likewise, another study disclosed greater potency of tedizolid up to 4–8 folds when compared with linezolid [32]. In a previous work by Bensaci and Sahm[33], 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[34]. On the other hand, a previous study conducted in Korea, described high linezolid resistance amongst *S. aureus* isolates (14.8%)[35]. 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[36, 37].

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[38]. 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[39]. Also, these findings support results from previous studies conducted in Egypt[34, 40].

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.

REFERENCES

1. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. Front Cell Infect Microbiol. 2020; 10: 107. https://doi.org/10.3389/fcimb.2020.00107
2. Haaber J, Penades JR, Ingmer H. Transfer of antibiotic resistance in *Staphylococcus aureus*. Trends Microbiol. 2017; 25: 893–905. https://doi.org/10.1016/j.tim.2017.05.011
3. Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz R, et al. Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. Genome Biol. 2017; 18(1): 130. https://doi.org/10.1186/s13059-017-1252-9
4. Zhang P, Fu Y, Zhao H, Liu X, Wu X, Lin T, et al. Dynamic insights into increasing antibiotic resistance in *Staphylococcus aureus* by label-free SERS using a portable Raman spectrometer. Spectrochim Acta A Mol Biomol Spectrosc. 2022; 273: 121070. https://doi.org/10.1016/j.saa.2022.121070
5. Dhungel S, Rijal KR, Yadav B, Dhungel B, Adhikari N, Shrestha UT, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence, Antimicrobial Susceptibility Pattern, and Detection of *mec*A Gene among Cardiac Patients from a Tertiary Care Heart Center in Kathmandu, Nepal. Infect Dis. 2021; 14: 11786337211037355. https://doi.org/10.1177/11786337211037355
6. Abad L, Tafani V, Tasse J, Josse J, Chidiac C, Lustig S, et al. Evaluation of the ability of linezolid and tedizolid to eradicate intraosteoblastic and biofilm-embedded *Staphylococcus aureus* in the bone and joint infection setting. J Antimicrob Chemother. 2019; 74(3): 625–632. https://doi.org/10.1093/jac/dky473
7. Tayal A, Singh NP, Rai S, Gupta K, Gupta A, Agarwal AN, et al. First study on detection of cryptic resistance to linezolid among clinical isolates of methicillin resistant *Staphylococcus aureus* from India. Indian J Med Microbiol. 2022. https://doi.org/10.1016/j.ijmmb.2022.05.004
8. Ruiz-Ripa L, Feßler AT, Hanke D, Eichhorn I, Azcona-Gutiérrez JM, Alonso CA, et al. Mechanisms of Linezolid Resistance Among Clinical *Staphylococcus* spp. in Spain: Spread of Methicillin- and Linezolid-Resistant *S. epidermidis* ST2. Microb Drug Resist. 2020. https://doi.org/10.1089/mdr.2020.0122

9. Hasannejad-Bibalan M, Mojtahedi A, Biglari H, Halaji M, Ebrahim-Saraie HS. Antibacterial activity of tedizolid, a novel oxazolidinone against methicillin-resistant *Staphylococcus aureus*: A systematic review and meta-analysis. Microb Drug Resist. 2019; 25(9): 1330−1337. https://doi.org/10.1089/mdr.2018.0457
10. Zhanel GG, Love R, Adam H, Golden A, Zelenitsky S, Schweizer F, et al. Tedizolid: A Novel Oxazolidinone with Potent Activity Against Multidrug-Resistant Gram-Positive Pathogens. Drugs. 2015; 75: 253−270. https://doi.org/10.1007/s40265-015-0352-7
11. Hori T, Owusu YB, Sun D. US FDA-Approved Antibiotics During the 21st Century. Infect Immun. 2022; 556−585. https://doi.org/10.1016/B978-0-12-818731-9.00144-0
12. Winn WC, Allen SD, Janda WM, Koneman E, Procop G, Schreckenberger P. et al. Introduction to microbiology part II: Guidelines for the Collection, Transport, Processing, Analysis and Reporting of Cultures from Specific Specimen Sources. In: Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW Eds. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed., Philadelphia: Lippincott Williams & Wilkins. 2006; 67−105.
13. Kateete DP, Kimani CN, Katabazi FA, Okeng F, Okee MS, Nanteza A, et al. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob. 2010; 9(1): 1−7. https://doi.org/10.1186/1476-0711-9-23
14. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Clinical and Laboratory Standards Institute (CLSI). 2020; Wayne, PA, USA.
15. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Eleventh Edition: M07. Clinical and Laboratory Standards Institute (CLSI). 2018; Wayne, PA, USA.
16. EUCAST. European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints. 2022; Version 12.0. http://www.eucast.org.
17. Adwan K. Fast DNA isolation and PCR protocols for detection of methicillin-resistant *staphylococci*. Folia Microbiol. 2014; 59:5−8. https://doi.org/10.1007/s12223-013-0259-1
18. Shrief R, El-Kholy RM, Rizk MA, Zaki MS. Prevalence of Tetracycline Resistant Genes in *Staphylococcus aureus* Isolates from Surgical Site Infections Egypt. Biosci Biotechnol Res Asia. 2019; 16(2): 221−228. http://doi.org/10.13005/bbra/2739
19. Morales G, Picazo JJ, Baos E, Candel, FJ, Arribi A, Peláez B, et al. Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. Clin Infect Dis. 2010; 50(6):821−5. https://doi.org/10.1086/650574
20. Gan T, Shu G, Fu H, Yan Q, Zhang W, Tang H, et al. Antimicrobial resistance and genotyping of *Staphylococcus aureus* obtained from food animals in Sichuan Province, China. BMC Vet Res. 2021; 17(1): 177. https://doi.org/10.1186/s12917-021-02884-z
21. Narula H, Chikara G, Gupta P. A prospective study on bacteriological profile and antibiogram of postoperative wound infections in a tertiary care hospital in Western Rajasthan. J Family Med Prim Care. 2020; 9(4):1927−1934. https://doi.org/10.4103/jfmpc.jfmpc_1154_19
22. Alfeky AAE, Tawfick MM, Ashour MS, El-Moghazy AA. High Prevalence of Multi-drug Resistant Methicillin-Resistant *Staphylococcus aureus* in Tertiary Egyptian Hospitals. J Infect Dev Ctries. 2022; 16(05): 795−806. https://doi.org/10.3855/jidc.15833
23. Shariati A, Dadashi M, Moghadam MT, Belkum AV, Yaslianifard S, Darban-Sarokhalil D. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis. Sci Rep. 2020; 10: 12689. https://doi.org/10.1038/s41598-020-69058-z
24. ElSayed N, Ashour M, Amine AEK. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt. Germs. 2018; 8(3):134−139. https://doi.org/10.18683/germs.2018.1140
25. Ahmed EF, Gad GF, Abdalla AM, Hasaneen AM, Abdelwahab SF. Prevalence of methicillin resistant *Staphylococcus aureus* among Egyptian patients after surgical interventions. Surg Infect. 2014; 15(4): 404−411. https://doi.org/10.1089/sur.2013.212
26. Abdel-Maksoud M, El-Shokry M, Ismail G, Hafez S, El-Kholy A, Attia A, et al. Methicillin-resistant *Staphylococcus aureus* recovered from healthcare-and community-associated infections in Egypt. Int J Bacteriol. 2016; 2016: 5751785. http://doi.org/10.1155/2016/5751785
27. Hamada M, Yamaguchi T, Sato A, Ono D, Aoki A, Kajiwara C, et al. Increased incidence and plasma-biofilm formation ability of SCC mec type IV methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients with

bacteremia. Front Cell Infect Microbiol. 2021; 11: 602833. https://doi.org/10.3389/fcimb.2021.602833

28. Montazeri EA, Khosravi AD, Jolodar A, Ghaderpanah M, Azarpira S. Identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from burn patients by multiplex PCR. Burns. 2015; 41(3): 590−594. https://doi.org/10.1016/j.burns.2014.08.018
29. Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018; 31(4): e00020−18. https://doi.org/10.1128/CMR.00020-18
30. Múnera JMV, Ríos AMO, Urrego DM, Quiceno JNJ. In vitro susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from skin and soft tissue infections to vancomycin, daptomycin, linezolid and tedizolid. Braz J Infect Dis. 2017; 21: 493−499. https://doi.org/10.1016/j.bjid.2017.03.010
31. Livermore DM, Mushtaq S, Warner M, Woodford N. Activity of oxazolidinone TR-700 against linezolid-susceptible and-resistant *staphylococci* and *enterococci*. J Antimicrob Chemother. 2009; 63(4): 713−715. https://doi.org/10.1093/jac/dkp002
32. Barber KE, Smith JR, Raut A, Rybak MJ. Evaluation of tedizolid against *Staphylococcus aureus* and *enterococci* with reduced susceptibility to vancomycin, daptomycin or linezolid. J Antimicrob Chemother. 2016; 71(1): 152−155. https://doi.org/10.1093/jac/dkv302
33. Bensaci M, Sahm D. Surveillance of tedizolid activity and resistance: in vitro susceptibility of Gram-positive pathogens collected over 5 years from the United States and Europe. Diagn Microbiol Infect Dis. 2017; 87(2): 133−138. https://doi.org/10.1016/j.diagmicrobio.2016.10.009
34. Rania Abd El-Hamid El-Kady, Medhat Abd El-Massih El-Daker, Mona Abbas Ali. The Multi-drug Resistance *cfr* Gene: an Evolving Mechanism for Linezolid Resistance in *Staphylococcus aureus*. Egypt J Med Microbiol. 2017; 26 (4): 67−72
35. Yoo IY, Kang OK, Shim HJ, Huh HJ, Lee NY. Linezolid resistance in methicillin-resistant *Staphylococcus aureus* in Korea: High rate of false resistance to linezolid by the VITEK 2 system. Ann Lab Med. 2020; 40(1):57−62. https://doi.org/10.3343/alm.2020.40.1.57
36. Betriu C, Morales G, Rodríguez-Avial I, Culebras E, Gómez M, López-Fabal, F, et al. Comparative activities of TR-700 (torezolid) against staphylococcal blood isolates collected in Spain. Antimicrob Agents Chemother. 2010; 54(5): 2212−2215. https://doi.org/10.1128/AAC.01653-09
37. Locke JB, Zurenko GE, Shaw KJ, Bartizal K. Tedizolid for the management of human infections: in vitro characteristics. Clin Infect Dis. 2014; 58(1): S35−S42. https://doi.org/10.1093/cid/cit616
38. Rodríguez-Noriega E, Hernández-Morfin N, Garza-Gonzalez E, ocanegra-Ibarias P, Flores-Treviño S, Esparza-Ahumada S, et al. Risk factors and outcome associated with the acquisition of linezolid-resistant *Enterococcus faecalis*. J Glob Antimicrob Resist. 2020; 21: 405−409. https://doi.org/10.1016/j.jgar.2020.01.010
39. Boudet A, Jay A, Dunyach-Remy C, Chiron R, Lavigne JP, Marchandin H. In-host emergence of linezolid resistance in a complex pattern of toxic shock syndrome toxin-1-positive methicillin-resistant Staphylococcus aureus colonization in siblings with cystic fibrosis. Toxins. 2021; 13(5): 317. https://doi.org/10.3390/toxins13050317
40. Abdelkhalek M, Elfeky A, Abo Elnasr L, Abd-Elmonsef M. Linezolid Resistance in Tanta University Hospitals: a Cross-sectional Study. Egypt J Med Microbiol. 2021; 30(4): 85−91. https://doi.org/10.21608/ejmm.2021.197466