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ORIGINAL ARTICLE

Emergence of resistance to last-resort antibiotics in clinical isolates of staphylococci with special reference to daptomycin and linezolid

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ABSTRACT

Background*: Treatment of staphylococcal infections has been complicated by the continuous emergence of antibiotic-resistant strains.* ***Objective:*** *In this study, we investigated the resistance pattern of clinical isolates of both coagulase-positive and coagulase-negative staphylococci to antibiotics recently introduced to treat staphylococcal infections.* ***Methodology****: Minimum inhibitory concentrations of antibiotics were determined by agar dilution or broth microdilution method. Identification of daptomycin- and linezolid-resistant isolates was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Moreover, the mechanism of resistance to daptomycin and linezolid was investigated on the molecular basis using PCR and amplicon sequencing.* ***Results****: Out of 104 staphylococci, 9 were resistant to ciprofloxacin (8.7%), 68 to clindamycin (65.4%), 2 to daptomycin (1.9%), 54 to erythromycin (51.9%), 6 to linezolid (5.8%), 3 to nitrofurantoin (2.9%), 75 to oxacillin (72.1%), 37 to teichoplanin (35.6%), 69 to tetracycline (66.3%), 51 to tigecycline (49%), and 41 to vancomycin (39.4%). Identification of daptomycin- and linezolid-resistant isolates revealed that they belong to Staphylococcus aureus, S. hominis, S. capitis, and S. epidermidis. In addition, sequencing of the mprF gene conferring daptomycin resistance revealed L431F and S829L point mutations in the two resistant isolates identified as S. aureus and S. hominis, respectively. Furthermore, linezolid resistance was due to optrA gene in two, and cfr in three of the resistant isolates.* ***Conclusion****: Resistance to the last-resort antibiotics used to treat staphylococcal infection has emerged. Therefore, the use of daptomycin and linezolid should be restricted to critical cases not susceptible to other available agents.*

INTRODUCTION

Staphylococci are Gram-positive and catalase-positive spherical bacteria with great clinical significance in human. They are opportunistic pathogens which inhabit the skin and mucous membranes. They can also disseminate to body organs through damaged skin to cause deep-seated infections for instance bacteraemia, osteomyelitis, pneumonia, and encephalitis[1]. Based on their capacity to coagulate citrated plasma, staphylococci can be differentiated into coagulase-positive staphylococci (CoPS) such as *S. aureus*, *S. intermedius* and *S. hyicus*, and coagulase-negative staphylococci (CoNS) such as *S. epidermidis*, *S. capitis*, and *S. hominis*. Among all staphylococci, *S. aureus* is the most abundant species linked with human infections. However, CoNS are also cause for concern as they have been linked to nosocomial infections particularly in immunocompromised individuals[2-4].

Management of infections caused by staphylococci has been complicated by the constant emergence of non-susceptible strains[5, 6]. Most staphylococci recovered from clinical sources were found non-susceptible to methicillin and, generally, resistant to several other classes of antibiotics especially penicillins, cephalosporins, quinolones, macrolides, and aminoglycocides[7, 8]. The traditional approach is to use vancomycin as a first-line therapy for methicillin-resistant strains of both CoPS and CoNS. Vancomycin interferes with the cell wall formation in target bacteria by binding to and inactivating essential precursors involved in the biosynthesis of cell wall at the division septum. However, evolution of vancomycin non-susceptibility in staphylococci has been reported[9]. Vancomycin resistance occurs when the bacterial cell wall becomes thickened due to mutations, providing protective barrier that prevents access of the antibacterial agent to its target. This situation has lead to an increased utilization of last-resort antibiotics for the management of staphylococcal diseases, particularly daptomycin, and linezolid[10].

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Daptomycin is a bactericidal lipopeptide antibiotic with outstanding activity against Gram-positive bacteria. This antibiotic was introduced in clinical use in 2004. Since then, it has been utilized as a last-line agent in the treatment of severe cases such as those caused by methicillin-resistant *S. aureus* (MRSA)[11]. It exerts its effect by inserting itself into the cytoplasmic membrane of target bacterial cells in a calcium-dependent way and thereby causes membrane depolarization, efflux of potassium ions, and eventually cell death[12]. Linezolid is another last-resort therapeutic alternative for treating infections due to multidrug-resistant staphylococci. It belongs to the oxazolidinone class of antibacterial agents and was approved in 2000 for treating pneumonia and skin infections due to MRSA[13]. Linezolid exhibits broad spectrum of bacteriostatic action by blocking bacterial protein synthesis through interfering with the 23S rRNA of the 50S ribosomal subunit[14].

The aim of the present study was to investigate the susceptibility of both CoPS and CoNS isolated from clinical sources to the last-resort antibiotics used to treat staphylococcal infections. The molecular mechanism of resistance was also explored in daptomycin- and linezolid-resistant isolates.

METHODOLOGY

Staphylococcal isolates:

We collected a total of 104 clinical isolates of staphylococci from Kasr Al-ainy hospital over a six-month period (between March and August 2019). Preliminary identification of isolates was based on phenotypic characteristics such as growth on mannitol salts agar (Oxoid), colony and cell morphology, Gram stain, and catalase production. Pure cultures were stored in tryptone soya broth, TSB (Oxoid) with 20 % (v/v) glycerol at – 80 ºC. Tube coagulase test was used to differentiate between CoPS and CoNS isolates[15]. *S. aureus* ATCC 29737 and *S. epidermidis* ATCC 35984 reference strains were used as positive and negative controls, respectively.

Determination of minimum inhibitory concentrations:

Eleven antibiotics used in treating staphylococcal infections were assessed in this study. These included ciprofloxacin, clindamycin, daptomycin, erythromycin, linezolid, nitrofurantoin, oxacillin, teichoplanin, tetracycline, tigecycline, and vancomycin. MIC values were determined by the agar dilution method on Mueller-Hinton Agar, MHA (Oxoid) based on the guidelines of the Clinical and Laboratory Standards Institute, CLSI[16] except for daptomycin. Daptomycin was tested by the broth microdilution method on cation-adjusted MH broth (CAMHB) supplemented with 50 µg/ml calcium[16]. For testing oxacillin, 2% NaCl was added to the medium. Plates were prepared with a concentration range of 512- 0.125 µg/ml of each antibiotic. Test bacteria were inoculated on the agar surface in 2-µl volumes containing about 10^4 CFU. For daptomycin, the concentration range was made up in 200-µl volumes in 96-well plates and inoculated with 10^4 CFU/well of test bacteria. Plates were then incubated at 37º C for 20 h. After incubation, MICs were read as the minimal concentration of the antibiotic at which no bacterial growth was detected. Interpretation of results was based on the MIC breakpoints of the CLSI[16] except for tigecycline for which MIC breakpoints of the British Society for Antimicrobial Chemotherapy, BSAC[17] were used.

Identification of resistant isolates:

Staphylococcal isolates showing phenotypic resistance to daptomycin or linezolid were identified to the species level using matrix-assisted laser desorption ionization-time of flight mass spectrometry, MALDI-TOF MS (MALDI Biotyper®, Bruker, Germany) according to the manufacturer′s instructions.

Identification of resistance mechanism:

The mechanisms underlying resistance to daptomycin and linezolid was explored by a molecular approach. The *mprF* gene was amplified from the two daptomycin-resistant isolates by PCR and the amplicons were sequenced to determine possible mutations. The presence of *optrA* or *cfr* genes conferring linezolid resistance was also investigated in the six linezolid-resistant isolates. Oligonucleotide primers designed for the amplification of *mprF*, *optrA*, and *cfr* are shown in Table 1. Total DNA was prepared from bacterial cultures grown in TSB. The PCR reaction was set up as follows: 5 µl of DNA, 1 µM of each primer, 10 µl of thermopol buffer, 2 µl of $MgSO_4$, 2 units of Vent® DNA polymerase (New England Biolabs, UK) and 50 µM of dNTPs (Promega). The reaction volume was made up to 100 µl using sterile dH_2O. The reactions were primarily heated to 95 ºC for 5 min and run through 40 cycles of (95 ºC for 30 s, 60 ºC for 30 s, 72 ºC for 1 min), and then heated to 72 ºC for 5 min in a PCR machine (SensoQuest GmbH, Germany). The amplified fragments were resolved by gel electrophoresis on 1 % agarose. The two purified *mprF* gene samples were sent to Sigma-Scientific Co. (Cairo, Egypt) for sequencing. DNA sequencing was performed according to the dideoxy method[18]. The obtained sequences were compared to *mprF* sequence using BLAST[19] at the NCBI (https://www.ncbi.nlm.nih.gov/) and mutations determined.

Table 1: Oligonucleotide primers used in this study

Gene	Primer	Nucleotide sequence (5'-3')	Amplicon size (bp)	Reference
mprF	Forward	ATGAATCAGGAAGTTAAAAACA	2523	This study
	Reverse	TCCAAGCGCTTCAGGCATAA		
optrA	Forward	AGGTGGTCAGCGAACTAA	1395	20
	Reverse	ATCAACTGTTCCCATTCA		
cfr	Forward	TGAAGTATAAAGCAGGTTGGGAGTCA	746	21
	Reverse	ACCATATAATTGACCACAAGCAGC		

Nucleotide sequence accession numbers:

The nucleotide sequences of the complete coding region of the *mprF* gene amplified from *S. aureus* isolate STAPH64 (YM2) and *S. hominis* isolate STAPH101 (YM3) showing mutations that resulted in daptomycin non-susceptibility were deposited in the GenBank Data Library under accession numbers MW387039 and MW387040, respectively.

RESULTS

Susceptibility of isolates to tested antibiotics:

The tube coagulase test results revealed that out of the 104 isolates preliminarily identified as staphylococci, 55 were CoPS and 49 were CoNS. We tested the susceptibility of isolates to the eleven tested antibiotics by MIC determination. The MIC values (range, MIC_{50} and MIC_{90}) for tested antibiotics are shown in Table 2. Data revealed that the MIC values for antibiotics were generally higher in the CoPS subset of test bacteria than those in the CoNS group. Data also showed that among the newly introduced antibiotics for staphylococcal infections, daptomycin resistance with MIC value of 4 µg/ml was detected. Moreover, higher MIC values for linezolid (32 µg/ml), nitrofurantoin (256 µg/ml), and vancomycin (32 µg/ml) were also recorded among test bacteria (Table 2).

Table 2: MIC of tested antibiotics against 104 clinical staphylococcal isolates

Antibiotic	Minimum Inhibitory Concentration (µg/ml)					
	CoPS (n=55)			CoNS (n=49)		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Ciprofloxacin	0.5- 8	1	2	0.125- 8	1	2
Clindamycin	0.125- 64	8	64	0.125- 64	8	16
Daptomycin	0.125- 4	0.25	1	0.125- 4	0.25	0.5
Erythromycin	0.25- 128	16	32	0.25- 64	16	32
Linezolid	0.5- 32	4	4	0.25- 16	4	4
Nitrofurantoin	2- 256	32	64	2- 128	32	32
Oxacillin	0.5- >256	64	256	0.25- >256	32	128
Teichoplanin	0.25- 32	4	32	0.25- 32	8	16
Tetracycline	1- 256	128	256	1- 128	64	64
Tigecycline	0.125- 8	0.5	4	<0.125- 4	0.25	4
Vancomycin	0.25- 32	16	16	0.125- 32	16	32

CoPS, coagulase-positive staphylococci; CoNS, coagulase-negative staphylococci. MIC_{50} and MIC_{90}, MIC value at which ≥ 50% and ≥90% of the isolates are inhibited, respectively.

Data also revealed that resistance to oxacillin was the most common among tested isolates followed by resistance to tetracycline, clindamycin, erythromycin, and tigecycline. On the other hand, most of isolates were susceptible to daptomycin, nitrofurantoin, linezolid, and ciprofloxacin (Figure 1).

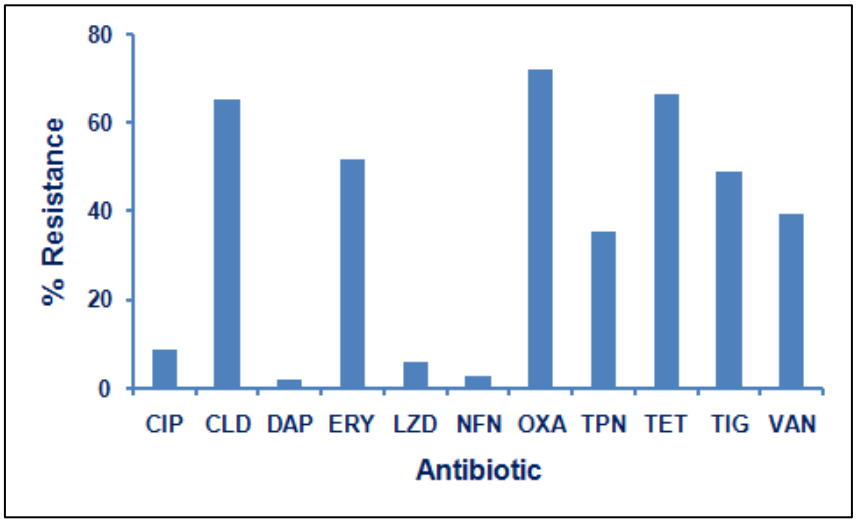

Fig. 1: Prevalence of antibiotic resistance in 104 clinical staphylococci isolates. CIP, ciprofloxacin; CLD, clindamycin; DAP, daptomycin; ERY, erythromycin; LZD, linezolid; NFN, nitrofurantoin; OXA, oxacillin; TPN, teichoplanin; TET, tetracycline; TIG, tigecycline; VAN, vancomycin.

Confirmation of identity and characterization of resistant isolates:

We further identified the isolates showing phenotypic non-susceptibility to either daptomycin or linezolid using MALDI-TOF MS. The two daptomycin-resistant isolates; STAPH64 and STAPH101 were identified as *S. aureus* and *S. hominis*, respectively. For GenBank submission purposes, these two daptomycin-resistant isolates were designated as *S. aureus* strain YM2 and *S. hominis* strain YM3. Both strains had daptomycin MIC of 4 µg/ml and were, in addition, resistant to multiple other antibiotics (Table 3). The six linezolid-resistant isolates were also multidrug resistant and belonged to three staphylococcal species; *S. capitis*, *S. aureus*, and *S. epidermidis* with linezolid MIC range of 8- 32 µg/ml (Table 3).

Table 3: Characterization of daptomycin and linezolid resistant isolates

Isolate	Identification	MIC (µg/ml)	Resistance phenotype
Daptomycin-resistant			
STAPH64 (YM2)	*S. aureus*	4	CIP, CLD, ERY, OXA, TPN, TET, TIG, VAN
STAPH101(YM3)	*S. hominis*	4	CLD, ERY, OXA, TPN, TET, VAN
Linezolid-resistant			
STAPH18	*S. capitis*	16	CLD, ERY, OXA, TET, TIG
STAPH31	*S. aureus*	32	ERY, OXA, TPN, TET, TIG, VAN
STAPH38	*S. aureus*	8	CLD, ERY, OXA, TPN, TET, TIG, VAN
STAPH86	*S. epidermidis*	8	CIP, CLD, OXA, TET, TIG, VAN
STAPH92	*S. aureus*	16	CLD, ERY, OXA, TET
STAPH99	*S. aureus*	32	CIP, CLD, ERY, OXA, TPN, TET, VAN

CIP, ciprofloxacin; CLD, clindamycin; ERY, erythromycin; OXA, oxacillin; TPN, teichoplanin; TET, tetracycline; TIG, tigecycline; VAN, vancomycin.

Molecular characterization of resistant isolates:

To characterize the molecular mechanism of daptomycin non-susceptibility, the *mprF* was amplified from the two daptomycin-resistant isolates by PCR and the amplicons were sequenced to detect any potential mutations linked to daptomycin resistance. The gene sequences were then aligned to the reference staphylococcal *mprF* gene using the NCBI BLAST. Results revealed that daptomycin resistance was due to mutations in the nucleotide sequence of the *mprF* gene, which resulted in point mutations in the corresponding MprF protein. In isolate STAPH64 (*S. aureus* strain YM2), the leucine residue 431 was substituted with phenylalanine (L431F) while in isolate STAPH101 (*S. hominis* strain YM3), the serine residue 829 was replaced by leucine (S829L). The 2523-bp *mprF* gene amplified from strain YM2 and strain YM3 is shown in Figure 2A and the location of the detected point mutations in the MprF protein is shown in Figure 2B&C.

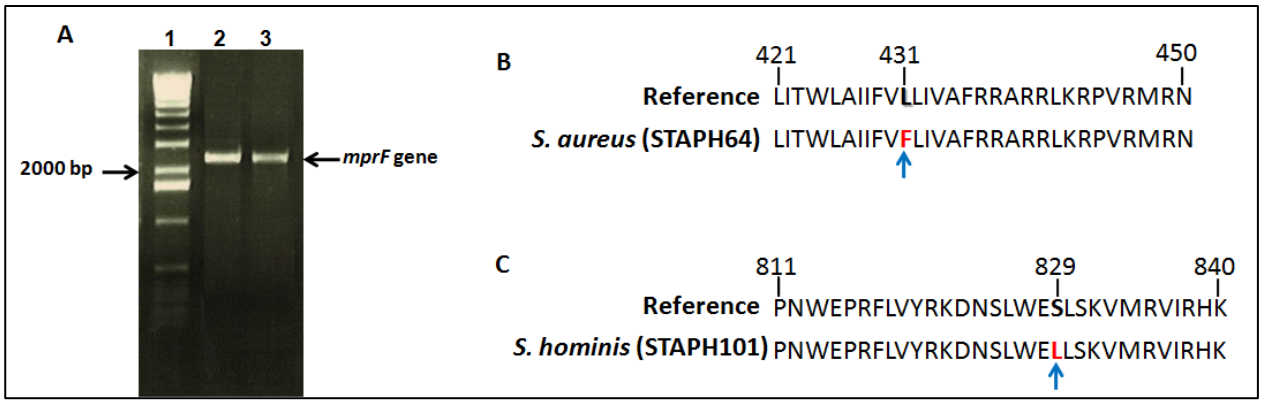

Fig. 2: Molecular characterization of daptomycin resistance. In (**A**), the 2523-bp *mprF* gene was amplified by PCR from daptomycin-resistant isolates STAPH64 (lane 2) and STAPH101 (lane 3). In (**B**), the amino acid sequence of STAPH64 MprF compared to the reference sequence showing L431F point mutation that resulted in daptomycin resistance. In (**C**), the amino acid sequence of STAPH101 MprF compared to the reference sequence showing S829L point mutation.

Moreover, we also explored the molecular mechanism of linezolid resistance in the six isolates showing phenotypic resistance to linezolid. The *optrA* and *cfr* genes associated with linezolid resistance were amplified by PCR. The *optrA* gene was detected in isolate STAPH18 (*S. capitis*) and isolate STAPH92 (*S. aureus*) while linezolid resistance was due to the presence of *cfr* gene in three *S. aureus* isolates (STAPH31, STAPH38 and STAPH99). We could not detect either *optrA* or *cfr* in *S. epidermidis* (isolate STAPH86) suggesting that linezolid resistance in this isolate could be due to different mechanism (Figure 3).

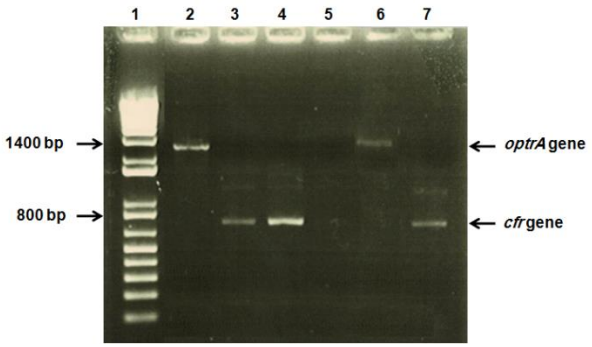

Fig. 3: Molecular characterization of linezolid resistance. The *optrA* and *cfr* genes were amplified by PCR from the six linezolid-resistant isolates. Lane 1, DNA ladder; lane 2, STAPH18 (*S. capitis*); lane 3, STAPH31 (*S. aureus*); lane 4, STAPH38 (*S. aureus*); lane 5, STAPH86 (*S. epidermidis*); lane 6, STAPH92 (*S. aureus*); lane 7, STAPH99 (*S. aureus*).

DISCUSSION

Antimicrobial resistance (AMR) represents a major public health problem worldwide. In a recent review on AMR, it was estimated that deaths due to multidrug-resistant pathogens could reach 10 million lives annually by the year 2050 unless action is taken[22]. In the fight against AMR, surveillance and prevalence studies are essential to the management of infectious diseases. Staphylococci are one of the most important bacterial species associated with hospital- and community-acquired infections. Treatment of staphylococcal diseases due to susceptible strains can be achieved by using β-lactams, tetracycline, clindamycin, or fuoroquinolones. However, most staphylococci are now resistant to these first-line therapeutic agents. The use of the glycopeptides; vancomycin and teichoplanin proved useful in multidrug-resistant staphylococci. Though, certain conditions such as bacteraemia and endocarditis due to non-susceptible strains usually require the utilization of the last-resort antibiotics; tigecycline, daptomycin, or linezolid[23, 24].

In the present study, we investigated the susceptibility of both CoPS and CoNS from clinical sources to antibiotics commonly used to treat staphylococcal diseases. Data suggested that resistance to oxacillin, tetracycline, and clindamycin was the most widespread among all tested isolates. This could be attributed to the massive use of these antibacterial agents in the clinical settings and the ease by which the resistance factors are exchanged between different species in mixed bacterial populations. Resistance to vancomycin (39.4%) was also alarming giving that vancomycin is the first-line therapy for MRSA infections. Results of vancomycin non-susceptibility

shown here are also similar to that of a recent study on CoNS reported by our research group[25]. Another finding of the present study that is a cause for concern is the prevalence of tigecycline resistance (49%) among tested isolates. Tigecycline is a member of a recently-introduced class of antibiotics known as glycylcyclines with activity against both Gram-positive and Gram-negative bacteria[26]. This antibiotic was approved in 2005 for treating skin infections and pneumonia[27]. Tigecycline is also considered an alternative therapy for MRSA infections. Tigecycline resistance that was conferred by the upregulation of efflux proteins due to alterations in *mepR* and *mepA* genes has been reported[28]. Despite the evolution of tigecycline resistance, the antibiotic was found highly effective (99.9%) on MRSA in a latest systematic review[11]. On the other hand, the prevalence of non-susceptibility to daptomycin, nitrofurantoin, linezolid, and ciprofloxacin in this study was the least among tested staphylococci. Therefore, these antibiotics can still be used to treat staphylococcal infections.

Given the significance of daptomycin and linezolid as last-resort antibiotics in the management of staphylococcal infections, it was important to verify the identity of non-susceptible isolates, to find out the molecular basis of resistance, and to assess the presence of transferable resistance elements in the clinical setting. Therefore, we adopted MALDI-TOF to identify the isolates and explored the molecular mechanisms underlying the daptomycin and linezolid non-susceptibility by PCR and amplicon sequencing. Perfect identification of resistant isolates was achieved using MALDI-TOF MS. This analytical technique has been widely used for the identification of clinical bacteria with high accuracy[29]. Results of the identification of daptomycin-resistant isolates STAPH64 and STAPH101 (MIC 4 μg/ml) revealed that STAPH64 was *S. aureus* and STAPH101 was *S. hominis*. Other studies have reported the evolution of daptomycin resistance in both *S. aureus* and CoNS especially *S. capitis*[6, 30]. In addition, the six linezolid-resistant isolates (MIC 8-32 μg/ml) were identified as *S. aureus* (4 isolates), *S. capitis* (1 isolate), and *S. epidermidis* (1 isolate). Similar results on linezolid-resistant strains of CoPS and CoNS were recently reported[31, 32]. The results of daptomycin and linezolid non-susceptibility presented here as well as those reported in previous studies suggest that the genetic entities conferring resistance to these agents can spread between different staphylococcal species. We then characterized the genetic basis of resistance in these isolates.

Daptomycin is a lipopeptide antibacterial agent that efficiently kills staphylococci. Current information on daptomycin resistance in staphylococci suggests that non-susceptibility results from different mutations in the genes involved in phospholipid biosynthesis. The most widespread gene, which is linked to daptomycin non-susceptibility in clinical strains is the *mprF* gene[33]. The *mprF* gene encodes for a bifunctional membrane protein phosphatidylglycerol lysyltransferase (also recognized as Multiple Peptide Resistance Factor, MprF). The MprF protein comprises three domains; an N-terminal flippase domain, a C-terminal synthase domain, and a central bifunctional domain[34]. Amino acid replacements linked to daptomycin resistance were observed in all three domains. Examples of these point mutations include G61V in the flippase domain, L826F in catalytic synthase domain, and T345I in the central domain[35]. Data presented here revealed an amino acid replacement in the C-terminal domain of the MprF protein of both resistant isolates (GenBank accession numbers MW387039 and MW387040). In STAPH64 isolate (*S. aureus* YM2), daptomycin resistance was due to the substitution of leucine residue 431 with phenylalanine (L431F) and in STAPH101 (*S. hominis* YM3), serine residue 829 was substituted with leucine (S829L). The S829L point mutation was reported before in a clinical isolate with daptomycin-resistant phenotype after extended treatment of endocarditis originally infected with a susceptible *S. aureus* strain[33]. This proposes that the same point mutation can occur in different staphylococcal strains.

Linezolid is the first licensed oxazolidinone with broad-spectrum activity against multidrug-resistant Gram-positive bacteria. Non-susceptibility to linezolid has been distinguished among both CoPS and CoNS soon after its deployment[36]. Linezolid resistance is conferred by mutations in domain V of the 23S rRNA or by the acquisition of resistance genes such as *cfr*, *optrA*, or *poxtA*[37]. The chloramphenicol-florfenicol resistance (*cfr*) gene codes for the enzyme 23S rRNA methyltransferase. The *cfr* gene confers multidrug resistance phenotype to oxazolidinone in addition to other antibacterial agents such as phenicols, lincosamides, pleuromutilins, and streptogramin A[38]. The *optrA* gene encodes an ATP-binding protein that confers non-susceptibility to oxazolidinones through ribosomal protection[39]. Recently, the *poxtA* gene was reported in MRSA strains to be associated with decreased susceptibility to oxazolidinones, phenicols, and tetracycline[40]. Molecular analysis of linezolid-resistant isolates in the present study revealed that *optrA* gene was found in two isolates identified as *S. capitis* and *S. aureus*. In addition, linezolid resistance was due to *cfr* in three *S. aureus* isolates while the *S. epidermidis* isolate had neither *optrA* nor *cfr* suggesting that linezolid resistance in this isolate could be due to other mechanism such as mutation in the 23S rRNA. The detection of transferable plasmid-borne genes such as *cfr* and *optrA* in the clinical isolates studied here is of great epidemiological significance as these genes could easily disseminate to other strains and species in clinical setting.

CONCLUSION

We herein report on the evolution of resistance to the last-resort antibiotics particularly daptomycin and linezolid in both coagulase-positive and coagulase-negative staphylococci from clinical sources. Identification of isolates showing phenotypic non-susceptibility to daptomycin and linezolid revealed that most of the resistant isolates were *S. aureus*. Moreover, resistance in *S. hominis*, *S. capitis*, and *S. epidermidis* was also detected. Molecular analysis of resistant isolates revealed that daptomycin resistance was due to point mutations; L431F and S829L in the MprF protein of *S. aureus* and *S. hominis*, respectively. Moreover, *optrA* and *cfr* genes were detected in linezolid-resistant isolates. Accordingly, we recommend restricting the use of these antibiotics to life-threatening conditions where the therapeutic use of other agents is not successful.

Authors' contributions:

- Yasser M. Ibrahim conceived and designed the study.
- Yasser M. Ibrahim, Nouran H. Assar, and Ahmed M. Abouwarda carried out the experiments.
- Yasser M. Ibrahim, Nouran H. Assar, and Ahmed M. Abouwarda analyzed the data.
- Yasser M. Ibrahim wrote the manuscript.
- Nouran H. Assar and Ahmed M. Abouwarda critically reviewed the manuscript.

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- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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