



Isolation and characterization of vancomycin and erythromycin resistant *Staphylococcus aureus* from Cairo, Egypt

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ABSTRACT

Aims: *Staphylococcus aureus* is an important opportunistic human pathogen. The emergence of macrolide and vancomycin resistant *S. aureus* is of great concern for treatment of *S. aureus* infections. The current study aimed to investigate the pattern of antibiotic resistance in *S. aureus* clinical isolates recovered from El Boos Students' hospital in Cairo, Egypt.

Methodology and results: Sixty unduplicated *S. aureus* isolates were recovered from El Boos Students' hospital in Cairo, Egypt for 11 months period. The antibiotic susceptibility test revealed that all isolates were resistant to eleven antibiotics, but only 49 *S. aureus* isolates were resistant to ceftiofuran. The minimum inhibitory concentrations (MIC) of both erythromycin and vancomycin were determined by broth microdilution method. Two methicillin resistant *S. aureus* (MRSA) isolates showing tolerance to both erythromycin and vancomycin at high concentration were selected for further characterization. One isolate was recovered from eye infection and had MIC at 256 µg/mL of both erythromycin and vancomycin. While another isolate was recovered from throat infection and had MIC of erythromycin and vancomycin up till 512 µg/mL. The presence of resistance genes (*ermA*, *ermB*, *ermC*, *mef*, *msrA*, *vanA* and *vanB*) were confirmed by polymerase chain reaction (PCR). Both MRSA isolates carried all tested resistance genes.

Conclusion, significance and impact of study: This study highlights the concern of presence of multidrug-resistant *S. aureus* which showed resistance to high concentrations of erythromycin, vancomycin and carried *ermA*, *ermB*, *ermC*, *mef*, *msrA*, *vanA* and *vanB* genes, therefore imposes risk of failure to treat such infections.

Keywords: *S. aureus*, erythromycin, vancomycin, Egypt

INTRODUCTION

Staphylococcus aureus, Gram positive (G +ve) cocci is one of the major health hazards with global impact. It causes a broad range of diseases such as skin, nosocomial, osteoarticular infections and septicemia (David and Daum, 2010; Goss and Muhlebach, 2011; Tong *et al.*, 2015). *Staphylococcus aureus* has been recognized as a worldwide disease causal agent and has become a major pathogen associated with both hospital and community-acquired infections (Tong *et al.*, 2015; Lake *et al.*, 2018).

Nowadays *S. aureus* strains have developed resistance to many commonly used antibiotics of groups, aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin), lincosamides (clindamycin) and tetracyclines (oxytetracycline) due to excessive and inappropriate use of antibiotics for human and animal treatment. In addition, *S. aureus* isolates are often found resistant to other beta-lactams (amoxicillin and

amoxicillin/clavulanate), cephalosporins (ceftiofuran, ceftriaxone and cefepime) and carbapenem (imipenem) (Dubey *et al.*, 2013).

This has led to use of macrolides and glycopeptides antibiotics, which represented a major alternative to beta-lactams for the treatment of infections caused by G +ve bacteria such as *S. aureus* (Roberts, 2008; Anstead *et al.*, 2014).

Macrolides antibiotics act by binding to 23S rRNA (ribosomal ribonucleic acid) in 50S ribosome subunit and thus inhibiting protein synthesis in bacterial cell (Romero *et al.*, 2011). *Staphylococcus aureus* resists macrolides antibiotics in three ways: (1) through target-site modification by methylation or mutation (encoded by *erm* genes), (2) through efflux of the antibiotic (encoded by *msrA/B* gene) and (3) by drug inactivation (encoded by *mphC* gene) (Petinaki and Papagiannitsis, 2018). Ribosomal methylation, in bacteria, encoded by *erm* genes are usually carried by plasmids and transposons. Four major classes of *erm* genes are detected in

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microorganisms: *ermA*, *ermB*, *ermC* and *ermF*. *ermA* and *ermC* typically are staphylococcal gene classes (Petinaki and Papagiannitsis, 2018). In G⁺ve organisms, active efflux is caused by two classes of pumps, ATP-binding-cassette transporter superfamily and major facilitator superfamily. The first determinant encoding ATP-binding-cassette transporter in staphylococci was the plasmid-borne *msrA* gene (Leclercq, 2002). Erythromycin resistance through efflux pump is also encoded by *mef* genes (de la Pedrosa *et al.*, 2008). Several subclasses of the *mef* gene have been described in streptococci including: *mef(A)* and *mef(E)* (more common) and the *mef(I)* and *mef(O)* (rarely encountered) (Klaassen and Mouton, 2005; Sangvik *et al.*, 2005; Mingoia *et al.*, 2007; Blackman Northwood *et al.*, 2009).

Vancomycin is a glycopeptide antibiotic agent used for treatment of severe *S. aureus* infections. *Staphylococcus aureus* exhibited two types of glycopeptide resistance: vancomycin-intermediate *S. aureus* (VISA) strains which had thickened cell wall, due to mutations in cell wall synthesis regulator genes (eg. *vraSR*, *saeSR* and *graSR*) and vancomycin-resistant *S. aureus* (VRSA) strains which acquire *vanA* or *vanB* genes. The *vanA* gene product is a ligase that produces D-Alanine (Ala)-D-Lactate (Lac), a substitution for D-Ala-D-Ala which is a building block for peptidoglycan synthesis (Amr and Al Gammal, 2017; Asadpour and Ghazanfari, 2019; EIFeky *et al.*, 2019).

Nowadays emergence of macrolides and vancomycin resistant *S. aureus* in health-care settings is a critical issue as it poses a threat for the treatment of *S. aureus* infections. This study was conducted to investigate the antibiotic resistance pattern in *S. aureus* clinical isolates collected from El Boos Students' hospital in Cairo, Egypt.

MATERIALS AND METHODS

Isolation and identification of *Staphylococcus aureus*

Staphylococcus aureus isolates were recovered from clinical samples collected from El Boos Students' hospital in Cairo, Egypt over a period of 11 months from November 2018 to September 2019. Clinical samples of *S. aureus* were isolated from blood, urine, throat swab, eye swab, wound swab and pus. *Staphylococcus aureus* isolates were identified by culturing on blood agar and mannitol salt agar media, Gram staining and standard biochemical tests including catalase, coagulase tests and DNase (deoxyribonuclease) test (Silverton and Anderson, 1961; Koneman *et al.*, 1992; Cheesbrough, 2000; Bannerman, 2003; Atlas and Snyder, 2011).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed using Kirby-Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Bauer *et al.*, 1966; CLSI, 2016). The antibiotics tested included amoxicillin (AMX, 20 µg), amoxicillin/clavulanate (AUG, 30 µg), cefepime (FEP, 30 µg), cefoxitin (FOX, 30

µg), ceftriaxone (CRO, 30 µg), ciprofloxacin (CIP, 5 µg), clindamycin (CLI, 2 µg), erythromycin (E, 15 µg), gentamicin (GEN, 10 µg), imipinem (IPM, 10 µg), oxytetracycline (OTC, 30 µg) and vancomycin (VAN, 30 µg). All media and antibiotic disks were purchased from Oxoid, UK.

Determination of minimal inhibitory concentration (MIC)

Minimal inhibitory concentration values of erythromycin and vancomycin were determined by broth microdilution method using Muller-Hinton broth. Standard antibiotics were purchased from Sigma-Aldrich, US. The procedures were performed according to Andrews (2001) and CLSI (2016). *Staphylococcus aureus* are considered erythromycin resistant if the MIC values ≥ 8 µg/mL and vancomycin resistant *S. aureus* (VRSA) if their MIC value is ≥ 16 µg/mL (CLSI, 2016).

Detection of resistance genes by PCR

Bacterial DNA was extracted using Quick-DNA™ Miniprep plus Kit, (Zymo research, USA) according to the manufacturer's instructions. Seven primer pairs (from Willowfort, Birmingham, UK) were used in PCR, including macrolides resistance genes (*ermA*, *ermB*, *ermC*, *mef* and *msrA*) and vancomycin resistance genes (*vanA* and *vanB*), as shown in Table 1. PCR was performed in 25 µL reaction mixture which included 12.5 µL Cosmo PCR red Master Mix (Willowfort), 1.25 µL of each primer (forward and reverse), 4 µL of DNA template and 6 µL nuclease free water. PCR was performed using DNA thermal cycler (Perkin Elmer Cetus). The amplification was performed using the following temperature profile: initial denaturation (94 °C for 5 min), 35 cycles of denaturation (94 °C for 1 min), annealing temperature (as in Table 1 for 1 min) and extension (72 °C for 1 min) and an additional extension step (72 °C for 5 min). PCR products were analyzed by electrophoresis with 1% agarose gel stained with 0.05% ethidium bromide. *Staphylococcus aureus* Sa16 was used as *ermA* positive control strain, *Enterococcus faecalis* EF123 was used as *ermB* positive control strain, *S. aureus* 7512166-1 was used as *ermC* positive control strain, *Streptococcus* sp. 99M116286 was used as *mef* positive control strain, *Enterococcus* sp. 2A1-6 was used as *msrA* positive control strain, *Enterococcus faecium* ATCC 51559 was used as *vanA* positive control strain and *Enterococcus faecalis* ATCC 51299 was used as *vanB* positive control strain. The reaction mixture containing all components except DNA template was used as negative control.

RESULTS AND DISCUSSION

A total of 60 *S. aureus* isolates were recovered from clinical samples. All 60 *S. aureus* isolates were resistant to amoxicillin, amoxicillin/clavulanate, cefepime, ceftriaxone, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipinem, oxytetracycline and vancomycin.

Table 1: Primers used for PCR amplification of the studied genes.

Target gene	Primer sequence (5'-3')	Product size (bp)	Annealing temperature	References
<i>ermA</i>	F5'-TATCTTATCGTTGAGAAGGGATT-3' R5'-CTACACTTGGCTTAGGATGAAA-3'	139	57 °C	(Zmantar <i>et al.</i> , 2011)
<i>ermB</i>	F5'-CTATCTGATTGTTGAAGAAGGATT-3' R5'-GTTTACTCTTGGTTTAGGATGAAA-3'	142	59 °C	(Zmantar <i>et al.</i> , 2011)
<i>ermC</i>	F5'-CTTGTTGATCACGATAATTTCC-3' R5'-ATCTTTTAGCAAACCCGATTTC-3'	190	55 °C	(Zmantar <i>et al.</i> , 2011)
<i>mef</i>	F5'-AGTATCATTAACTACTAGTGC-3' R5'-TTCTTCTGGTACAAAAGTGG-3'	348	51 °C	(Zmantar <i>et al.</i> , 2011)
<i>msrA</i>	F5'-TCCAATCATAGCACAAAATC-3' R5'-AATTCCTCTATTTGGTGGT-3'	163	50 °C	(Zmantar <i>et al.</i> , 2011)
<i>vanA</i>	F5'-CATGAATAGAATAAAAAGTTGCAATA-3' R5'-CCCCTTTAACGCTAATACGACGATCAA-3'	1030	62 °C	(Tiwari and Sen, 2006)
<i>vanB</i>	F5'-GTGACAAACCGAGGCGAGGA-3' R5'-CCGCCATCCTCCTGCAAAAAA-3'	433	61 °C	(Al-Amery <i>et al.</i> , 2019)

F: Forward; R: Reverse.

Out of these 60 *S. aureus* isolates, 49 *S. aureus* isolates were considered as MRSA as they were resistant to ceftioxin (these isolates were selected from a bigger sample population of *S. aureus* collected from November 2018 to September 2019). Similar results were reported where more than half of *S. aureus* isolates were resistant to all tested antibiotics (ceftioxin, ciprofloxacin, clindamycin, erythromycin and gentamycin) (EIFeky *et al.*, 2019). Moreover, in a previous report, all isolated *S. aureus* were resistant to amoxicillin, ceftioxin and ceftriaxone, most of isolates were resistant to ciprofloxacin and erythromycin (Radhakrishna *et al.*, 2013). It was deduced that most of *S. aureus* isolates were resistant to amoxicillin, amoxicillin/clavulanate, ciprofloxacin, erythromycin, gentamycin and tetracycline (Abd El-Moez *et al.*, 2011; Shibabaw *et al.*, 2014). In a recent Egyptian study, most of clinical *S. aureus* isolates showed resistance against tetracycline, gentamycin and ciprofloxacin, while less than half of isolates were resistant to erythromycin (Youssef *et al.*, 2021). It was found that most of isolated *S. aureus* were resistant to ceftioxin, ciprofloxacin and erythromycin, while less than 40% were resistant to gentamycin and tetracycline (Cabrera *et al.*, 2020). However, in previous reports it was found that most of *S. aureus* isolates were resistant to ceftriaxone, amoxicillin and tetracycline, while least resistant was against ciprofloxacin (Torimiro *et al.*, 2013).

Methicillin resistant *S. aureus* (MRSA) resistance towards erythromycin and vancomycin was confirmed based on MIC results. The MIC values of erythromycin and vancomycin for 49 MRSA isolates are summarized in Table 2. According to MICs values of the 49 MRSA isolates, 5 isolates (10.2%) were resistant to erythromycin with MIC 8 µg/mL, 2 isolates (4.1%) had MIC value of 16 µg/mL, 2 isolates (4.1%) had MIC value of 32 µg/mL, 7 isolates (14.3%) with MIC value 64 µg/mL, 9 isolates (18.4%) (n=9) had MIC 128 µg/mL, while 17 isolates (34.7%) with MIC value 256 µg/mL and 7 isolates (14.3%) had MIC value 512 µg/mL. Similar results were obtained in previous reports which recorded maximum MIC value

≥256 µg/mL for erythromycin resistant MRSA isolates (Vandendriessche *et al.*, 2011; Teodoro *et al.*, 2012; Sadeghi and Mansouri, 2013). Our results were higher than that reported in previous studies, which the reported MIC value of erythromycin resistant MRSA isolates was ≥128 µg/mL (Turkyilmaz *et al.*, 2010; Yildiz *et al.*, 2014; Gostev *et al.*, 2017). However, a higher MIC value was recorded in a previous study where more than half of erythromycin resistant *S. aureus* isolates had MIC 1024 µg/mL (Piatkowska *et al.*, 2012).

On the other hand, a 10.2% (n=5) of MRSA isolates were resistant to vancomycin with MIC value 16 µg/mL, 10.2% (n=5) of resistant isolates had MIC value 32 µg/mL, 10.2% (n=5) had MIC value 64 µg/mL, 28.6% (n=14) had MIC 128 µg/mL, while 13 isolates (26.5%) with MIC value 256 µg/mL and 7 isolates (14.3%) had MIC value 512 µg/mL as shown in Table 2. This percentage (14.3%) was higher than that reported in a previous Egyptian study where less than 6% of VRSA had MIC value 512 µg/mL (El-Banna *et al.*, 2015). However, our result was lower than that recorded in a previous study where half of VRSA had MIC value 512 µg/mL (Shekarabi *et al.*, 2017).

From these isolates two MRSA isolates showed high level of erythromycin and vancomycin resistance, one isolate (E-VMRSA48; recovered from eye swab) was resistant to both erythromycin and vancomycin with MIC value 256 µg/mL, while other isolate (E-VMRSA9; recovered from throat swab) was resistant to both erythromycin and vancomycin with MIC value 512 µg/mL. Similar to our results, Iranian study revealed that VRSA isolate, recovered from diabetic foot ulcer, had MIC value 512 µg/mL and was erythromycin resistant (Dezfulian *et al.*, 2012). A previous Egyptian study reported that ten VRSA, isolated from clinical samples, had MIC value ≥16 µg/mL and were resistant to erythromycin (Amr and Al Gammal, 2017).

From PCR analysis, E-VMRSA9 and E-VMRSA48 isolates carried macrolide resistance genes (*ermA*, *ermB*, *ermC*, *mef* and *msrA*) and vancomycin resistance

Table 2: MIC values of 49 erythromycin and vancomycin resistant MRSA isolates.

Methicillin resistant <i>S. aureus</i> (MRSA)	Site of isolation	MIC ($\mu\text{g/mL}$)		Antibiotic resistance phenotype
		E	VAN	
MRSA1	Wound swab	512	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA2	Eye swab	128	16	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA3	Blood	512	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA4	Blood	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA5	Blood	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA6	Blood	128	32	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA7	Blood	8	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA8	Throat swab	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
E-VMRSA9	Throat swab	512	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA10	Urine	128	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA11	Throat swab	16	32	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA12	Throat swab	8	16	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA13	Throat swab	16	16	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA14	Eye swab	64	32	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA15	Wound swab	512	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA16	Blood	256	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA17	Throat swab	512	64	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA18	Blood	64	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA19	Blood	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA20	Blood	64	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA21	Blood	128	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA22	Throat swab	512	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA23	Wound swab	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA24	Wound swab	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA25	Blood	8	16	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA26	Blood	128	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA27	Pus	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA28	Pus	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA29	Pus	512	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA30	Throat swab	64	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA31	Pus	64	64	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA32	Pus	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA33	Blood	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA34	Pus	256	64	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA35	Blood	64	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA36	Eye swab	128	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA37	Throat swab	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA38	Throat swab	8	32	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA39	Eye swab	32	64	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA40	Eye swab	256	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA41	Throat swab	32	32	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA42	Urine	64	64	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA43	Urine	128	128	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA44	Blood	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA45	Pus	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA46	Eye swab	128	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA47	Wound swab	128	512	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
E-VMRSA48	Eye swab	256	256	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN
MRSA49	Blood	8	16	AMX, AUG, FEP, FOX, CRO, CIP, CLI, E, GEN, IPM, OTC, VAN

MIC: Minimum inhibitory concentration, AMX: Amoxicillin, AUG: Amoxicillin/clavulanate, FEP: Cefepime, FOX: Cefoxitin, CRO: Ceftriaxone, CIP: Ciprofloxacin, CLI: Clindamycin, E: Erythromycin, GEN: Gentamicin, IPM: Imipenem, OTC: Oxytetracycline, VAN: Vancomycin.

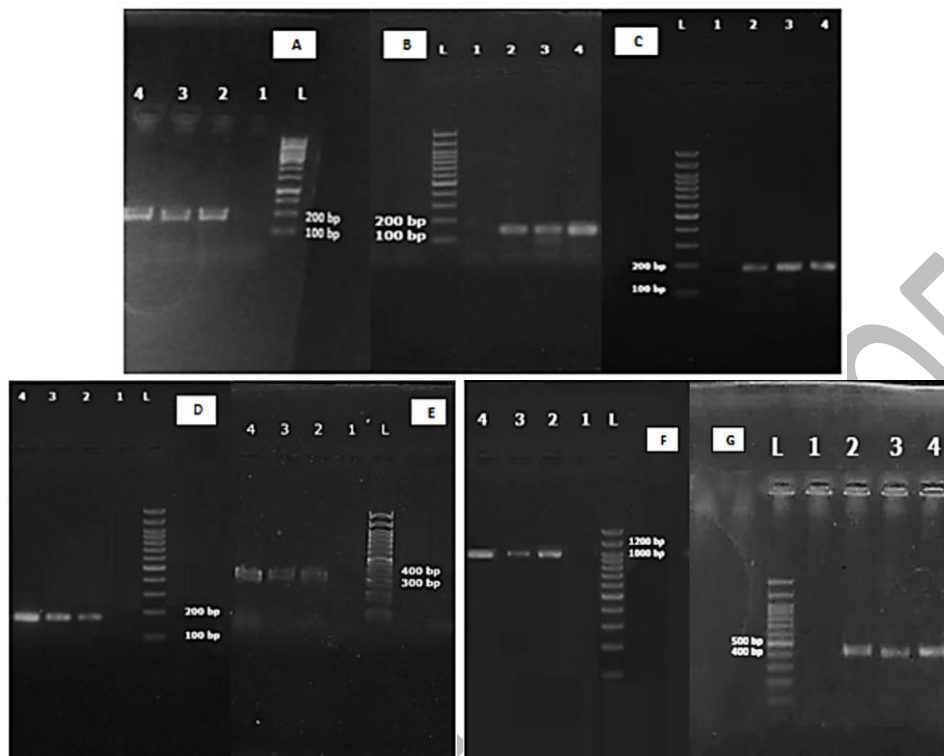


Figure 1: Amplified PCR products. A: Amplification of *ermA* gene (139 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. B: Amplification of *ermB* gene (142 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. C: Amplification of *ermC* gene (190 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. D: Amplification of *msrA* gene (163 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. E: Amplification of *mef* gene (348 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. F: Amplification of *vanA* gene (1032 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control. G: Amplification of *vanB* gene (433 bp). L, 100 bp DNA ladder; Lane 1, Negative-control; Lane 2, E-VMRSA9 isolate; Lane 3, E-VMRSA9 isolate; Lane 4, Positive-control.

genes (*vanA* and *vanB*). Similar results were reported in a previous study where two VRSA strain (MIC value 512 µg/mL) isolated from throat were resistant to erythromycin and carried resistance genes *ermA*, *ermB*, *msrA* and *vanA* (Shekarabi *et al.*, 2017). Figure 1 shows gel electrophoresis of amplified products of *ermA*, *ermB*, *ermC*, *mef*, *msrA*, *vanA* and *vanB* genes.

The findings of our study can be a warning about the dissemination of VRSA isolates in Egypt. To date, most of the reports revealed that VRSA strains were highly prevalent in America followed by Africa (3.6% and 2.5% respectively) (Shariati *et al.*, 2020). More studies are needed to explore the clonal diversity among VRSA isolates and the definite mechanisms by which these isolates acquire resistance to vancomycin. We urge further comprehensive molecular epidemiological surveillance studies on the extent and potential transmission of VRSA strains in humans in Egypt.

CONCLUSION

Isolates recovered from this study were resistant to most antibiotics currently in use. They were also resistant to high concentration of last-line drugs such as erythromycin and vancomycin, which limit the treatment options for physicians. More studies are still required to explore the mechanisms by which these multidrug resistant *S. aureus* isolates acquired tolerance to high concentrations of macrolides and vancomycin antibiotics which may help to overcome this problem and prevent spread of these isolates.

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