Journal of Antibiotics Research
Volume 3 | Issue 1
ISSN: 2574-5980

Research article Open Access

Molecular Characterization and Resistance Patterns of Multidrug Methicillin-Resistant *Staphylococcus aureus* Isolated from Wound

Abo-Amer AE[*1,2], Niaz AM[1] and Bamaga MS[3]

[1]Department of Biology, Faculty of Science, Division of Microbiology, University of Taif, Saudi Arabia
[2]Department of Botany and Microbiology, Faculty of Science, Division of Microbiology, Sohag University, Egypt
[3]Microbiology Lab, Al Hada Military Hospital, Taif, Saudi Arabia

[*]**Corresponding author:** Abo-Amer AE, Division of Microbiology, Department of Biology, Faculty of Science, Taif University, Taif (888), Saudi Arabia, Tel: +966 558209764, E-mail: a.aboamer@hotmail.com

Citation: Abo-Amer AE, Niaz AM, Bamaga MS (2019) Molecular Characterization and Resistance Patterns of Multidrug Methicillin-Resistant *Staphylococcus aureus* Isolated from Wound. J Antibioti Res 3(1): 101

Received Date: November 11, 2018 **Accepted Date:** August 24, 2019 **Published Date:** August 27, 2019

Abstract

Staphylococcus aureus is a serious risk to public health as it triggers human infections ranges from wound abscess to life threatening states. As involvement to the global effort the objectives of this study were to examine the incidence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Taif region, Saudi Arabia. Moreover, this work aimed to estimate the relation between the phenotypic antibiotic susceptibility patterns and the antibiotic resistance genes mong MRSA isolates. A total of 67 wound specimens were taken from patients. Methicillin-resistant *Staphylococcus aureus* (MRSA) was selected by growing on Mannitol Salt Agar supplemented with methicillin (5 mg/l). The prevalence of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant (MRSA) wound carriage among patients was 51 and 49 %, respectively. All MRSA isolated were resistant to penicillin, oxacillin and cefoxitin. However, 100% of MRSA isolates were determined as sensitive to linezolid, teicoplanin, vancomycin, tigecycline, mupirocin and rifampicin. 78% of MRSA isolates were multidrug resistant (MDR). The presence of resistance genes to methicillin (*mecA*) was mostly detected in the isolates. Macrolide-lincosamide-streptogramin B (*ermA*) gene was detected in all isolates. Tetracycline (*tetM*) gene was mostly found in the isolates. The genes responsible for tetracycline (*tetK*), Streptogramin A (*vatABC*) and macrolide-lincosamide-streptogramin B (*ermC*) were not detected. *mecA* gene was detected in 86% MRSA isolates. Most isolates carried a plasmid of > 10 kb. Some isolates appeared to be carried 4 plasmids with different sizes (2kb, 4kb, >10kb, >10kb). Linezolid, teicoplanin, vancomycin, mupirocin and rifampicin could be the best drugs of choice in treating MRSA infection. Moreover, MIC of vancomycin should be performed routinely to screen out vancomycin-intermediate *S. aureus* (VISA). Also, findings of this study might be helpful for developing an appropriate policy of hospital antibiotic administration to reduce the chances of *S. aureus* associated infections in Taif region.

Keywords: MRSA Wound Carriage; *mecA; aacA-aphD; ermAC; tetMK; vatABC*; Plasmids; 16S rRNA

Introduction

Staphylococcus aureus is a severe danger to public health as it causes human infections ranges from wound abscess to life threatening situations [1]. Emergence of antibiotics resistant *S. aureus* especially methicillin-resistant *S. aureus* (MRSA) is a global problem in both healthcare and community locations [2]. With the increase of MRSA associated infections, the use of glycopeptides (vancomycin and teicoplanin) are also rising gradually. The unreasonable and random use of glycopeptides causes in the emergence of even vancomycin resistance in *S. aureus*. Such resistance, however uncommon, but it is an emerging threat because of its possible to distribute rapidly [3]. Methicillin-resistant *S. aureus* are resistant to all penicillins, including semi synthetic penicillinase-resistant congeners, penems, carbapenems, and cephalosporins. The most important mechanism of resistance to penicillin is caused by the acquisition of an exogenous gene, *mecA*, that encodes an additional β-lactam-resistant penicillin-binding protein (PBP), termed PBP-2' (or PBP-2a). Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes β-lactamase [4]. The *aacA-aphD* gene, which encodes the bi-functional enzyme aminoglycoside-6-N-acetyltransferase/2-O-nucleotidyl transferase, mediates resistance to all clinically used aminoglycosides except streptomycin and neomycin [5]. The *ermA* and *ermC* genes are frequently responsible for the Macrolides, lincosamides and streptogramin B (MLSB) resistance in staphylococci coding for rRNA methylases, which can be either constitutive or inducible. Clinical *S. aureus* strains carrying *ermB* are rather infrequent [6]. For full resistance to the streptogramin combination quinupristin-dalfopristin, acetyltransferase *vatABC* genes are needed in bacterial strains to carry additional resistance to streptogramin A compounds [6]. The main mechanisms conferring resistance to tetracycline to bacteria are active efflux proteins, ribosomal protection proteins and enzymatic inactivation [7]. Molecular detection techniques

continue to increase in utility in clinical routine screening of *S. aureus* isolates to determine antimicrobial susceptibility patterns. MRSA can be transferred from person to person via skin or the sharing of contaminated items.

Antibiotic resistance is a significant threat to global health; it can affect anyone, of any age, in any country. Abuse of antimicrobial agents is an important cause of antibiotic resistance, which leads to increased medical expenses and mortality [8]. Therefore, the present study was aimed to investigate the incidence of MRSA in Taif region, Saudi Arabia. Also, it was aimed to investigate the prevalence resistance genes of methicillin (*mecA*), aminoglycoside (*aacA-aphD*), tetracycline (*tetKM*), streptogramin A (*vatABC*) and macrolide-lincosamide-streptogramin B (*ermAC*) among MRSA isolates.

Materials and Methods

Collection of Samples

Sixty seven samples of wounds were collected from patients at Taif province. Samples were taken by swabs and immediately transported to the laboratory and processed immediately or refrigerated at -20 ºC as soon as possible. The samples were taken by the staffs of Microbiology Labs of hospitals.

Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus*

The swabs were streaked on Mannitol Salt Agar plates (selective medium for *Staphylococcus aureus*). The plates were incubated for 24-48 hours at 37±1 ºC. The suspected *Staphylococcus* colonies-yellow colonies showing Mannitol fermentation were selected and subject to further characterization. Suspected colonies were stored in 40% glycerol at -70 ºC. Methicillin-resistant *Staphylococcus aureus* was distinguished from methicillin-sensitive *Staphylococcus aureus* (MSSA) by culturing on Mannitol Salt Agar supplemented with methicillin (5 mg/l). Mannitol Salt Agar with oxacillin (methicillin) is used for the isolation of methicillin-resistant *Staphylococcus aureus* from clinical specimens [9]. Morphological and biochemical characteristics of bacterial isolates were examined after incubation at 37 ºC for 24h. The bacterial isolates were distinguished according to Bergey's Manual of Systematic Bacteriology [10].

Antimicrobial Susceptibility and Minimal Inhibitory Concentrations (MICs) Tests

Kirby Bauer Test (disc diffusion method) was used [11]. A standard concentration of an organism was plated onto Mueller Hinton agar. Afterwards, paper discs containing fixed concentrations of antimicrobials were placed onto the surface of the media. Susceptibility is visualized by a zone of inhibition around the disc and the MICs were detected with different concentrations of antibiotics. The MICs were correlated to known MIC values of CLSI-defined breakpoints to determine whether the isolate was susceptible, intermediate, or resistant [12]. The followings antimicrobial agents were used: penicillin P, oxacillin OX, cefoxitin FOX, gentamicin GM, tobramycin TOB, levofloxacin LVX, moxifloxacin MXF, erythromycin E, clindamycin DA, linezolid LNZ, teicoplanin TEC, vancomycin VA, tetracycline TE, tigecycline TGC, fosfomycin FOS, nitrofurantoin NIT, fusidic Acid FA, mupirocin MUP, rifampicin RA and sulfamethoxazole/trimethoprim SXT.

Molecular Characterization of MRSA

Isolation of Chromosomal DNA: Isolation of chromosomal DAN from multidrug resistant MRSA isolates was performed by Thermo Scientific GeneJET Genomic DNA Purification Kit with some modifications. DNA samples were resolved by 1% agarose gel electrophoresis and then stained with ethidium bromide. Molecular weight ladder, 1Kb DNA ladder RTU (Ready-to- Use, GeneDirex) was employed.

16S rRNA Gene Analysis

Firstly PCR was achieved to amplify 16S rRNA gene. 20 µl-PCR reactions contained 2 µl of template DNA and 1X PCR master mix. Universal primers, 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (TAC GGY TAC CTT GTT ACG ACT T), were used. 35 amplification cycles were performed at 94 ºC for 45 s, 55 ºC for 60 s, and 72 ºC for 60 s. Amplified DNA fragments were ~ 1,400 bp. Unincorporated PCR primers and dNTPs were removed from PCR products by using Montage PCR Clean up kit (Millipore). PCR-products were resolved by 1% agarose gel electrophoresis and then stained with ethidium bromide. Molecular weight ladder, 1Kb DNA ladder RTU (Ready-to- Use, GeneDirex) was usedutilized. The purified PCR products of approximately 1,400 bp were sequenced by using two universal primers: 518F (CCA GCA GCC GCG GTA ATA CG) and 800R (TAC CAG GGT ATC TAA TCC). Sequencing was achieved by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were analyzed on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). Selected sequences of other microorganisms with greatest similarity to the 16S rRNA sequences of bacterial isolates were extracted from the nucleotide sequence databases and aligned using CLUSTAL W (1.81) Multiple Sequence Alignment generating phylogenetic tree.

Isolation of Plasmid DNA

Plasmid DNA was isolated from bacterial isolates by Thermo Scientific GeneJET Plasmid Miniprep Kit with some modifications.

Plasmid preparations were resolved by 1% agarose gel electrophoresis and then stained by ethidium bromide. Molecular weight marker, 1Kb DNA ladder RTU (Ready-to- Use, GeneDirex) were employed as a size standard.

Detection of Resistance Genes by Polymerase Chain Reaction (PCR)

The existence of genes related with resistance to Methicillin (*mecA*), Aminoglycoside (*aacA-aphD*), Tetracycline (*tetKM*), Streptogramin A (*vatABC*) and Macrolide-lincosamide-streptogramin B (*ermAC*) were verified by PCR. The set of primers used for each gene shown in Table 1 (Macrogen, Korea). GoTaq Green Master Mix kit (Promega, USA) was applied. PCR reactions were achieved in a total volume of 25 µl, containing 12.5 µl Master mix, 2 µl Forward Primer (10 pM), 2 µl Reverse Primer (10 pM), DNA Template 2 µl and 6.5 µl water. Amplification reactions were performed using a DNA thermocycler (Labnet International, Model: Multigene Opti Max) as follows: 95 ºC for 3 min, 35 cycles each involving of 94 ºC for 30 sec, 55 ºC for 30 sec and 72 ºC for30 sec, followed by a final extension step of 72 ºC for 4 min. Amplified PCR produts were resolved by 1% agarose gel electrophoresis and stained by ethidium bromide. Molecular weight ladder, 100 pb DNA ladder RTU (Ready-to-Use, GeneDirex), was used.

Target gene	Encoding	Oligonucleotide sequence (5'-3')	Amplicon size (bp)
aacA-aphD	Aminoglycoside resistance (GEN)	F: TAA TCC AAG AGC AAT AAG GGC R: GCC ACA CTA TCA TAA CCA CTA	227
tetK	Tetracycline resistance (OTE)	F: GTA GCG ACA ATA GGT AAT AGT R: GTA GTG ACA ATA AAC CTC CTA	360
tetM	Tetracycline resistance (OTE)	F: AGT GGA GCG ATT ACA GAA R: CAT ATG TCC TGG CGT GTC TA	158
vatA	Streptogramin A resistance (SYN)	F: TGG TCC CGG AAC AAC ATT TAT R: TCC ACC GAC AAT AGA ATA GGG	268
vatB	Streptogramin A resistance (SYN)	F: GCT GCG AAT TCA GTT GTT ACA R: CTG ACC AAT CCC ACC ATT TTA	136
vatC	Streptogramin A resistance (SYN)	F: AAG GCC CCA ATC CAG AAG AA R: TCA ACG TTC TTT GTC ACA ACC	467
ermA	Macrolide-lincosamide-streptogramin B resistance (ERY, CLI)	F: AAG CGG TAA ACC CCT CTG A R: TTC GCA AAT CCC TTC TCA AC	190
ermC	Macrolide-lincosamide-streptogramin B resistance (ERY, CLI)	F: AAT CGT CAA TTC CTG CAT GT R: TAA TCG TGG AAT ACG GGT TTG	299
mecA	Methicillin resistance (PEN, OXA)	F: AAA ATC GAT GGT AAA GGT TGG C R: AGT TCT GCA GTA CCG GAT TTG C	532
16S rDNA	Amplification control	F: CAG CTC GTG TCG TGA GAT GT R: AAT CAT TTG TCC CAC CTT CG	420

Table 1: The primer sequences and predicted sizes used in the PCR

Results

Isolation and Characterization of *Staphylococcus aureus*

Wound specimens were got from 67 patients. Of the 67 *Staphylococcus aureus* isolates tested, 33 (49%) were positive for MRSA by direct plating method on Minntol Salt Agar with methicillin. The MRSA isolates were designated as WO01, WO02, WO06, WO08, WO10, WO19, WO21, WO27, WO34, WO39, WO43, WO52, WO53, WO56, WO59, WO60, WO61, WO63, WO64, WO65, WO66, WO69, WO70, WO75, WO88, WO90, WO91, WO95, WO96, WO103, WO104, WO105 and WO112. All obtained isolates were characterized morphologically and biochemically as *Staphylococcus aureus. S. aureus* forms golden or white colonies on blood agar. All the isolates produce catalase, an enzyme which changes hydrogen peroxide (H_2O_2) to water and oxygen. The catalase test is suitable to differentiate *staphylococci* from *enterococci* and *streptococci*. Most of these strains ferment mannitol and trehalose anaerobically which, among other tests, may help to classify *S. aureus*.

Antibiotic Susceptibility Testing

Sixty seven isolates of *Staphylococcus aureus* isolated from wound swabs were screened for antimicrobial susceptibility test. The MICs of these bacterial isolates against different antimicrobial agents were determined (Table 2). The obtained results were interpreted according to CLSI guidelines 2016. As shown in Table 3, 49% of the isolates were detected as MRSA isolates. All MRSA isolates were resistant to penicillin, oxacillin and cefoxitin. However, 100% of MRSA isolates were determined as sensitive to linezolid, teicoplanin, vancomycin, tigecycline, mupirocin and rifampicin. All MRSA isolates were resistant to 3-11 antimicrobial agents. 78% of MRSA isolates were MDR. Most of MRSA isolates were MDR bacteria. MRSA isolates WO01, WO10, WO27, WO53, WO60, WO66 and WO70 were resistant to 10, 5, 7, 11, 10, 8 and 10 antimicrobial agents, respectively. Accordingly, these MDR-MRSA isolates were selected to further study

Isolates		MIC values (µg/ml)																		
	P	OX	FOX	GM	TOB	LVX	MXF	E	DA	LNZ	TEC	VA	TE	TGC	FOS	NIT	FA	MUP	RA	SXT
W01	≤0.5	≤4	≤8	16	16	4	1	8	8	1	0.5	1	16	0.12	8	16	32	8	0.5	320
W02	≤0.5	≤4	≤8	4	2	4	1	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	20
W06	≤0.5	≤4	≤8	0.5	1	0.5	0.25	0.25	0.25	2	0.5	0.5	1	0.12	8	16	1	2	0.5	10
W08	≤0.5	≤4	≤8	4	2	4	1	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	20
W10	≤0.5	≤4	≤8	8	4	4	2	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	160
W19	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	16	0.12	8	32	32	2	0.5	10
W21	≤0.5	≤4	≤8	8	2	4	2	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	320
W27	≤0.5	≤4	≤8	0.5	16	8	2	8	8	4	0.5	1	1	0.12	8	32	8	2	0.5	10
W34	≤0.5	≤4	≤8	16	8	0.25	0.25	0.25	0.25	2	0.5	0.5	16	0.25	8	16	16	2	0.5	10
W39	≤0.5	≤4	≤8	16	16	0.25	0.25	0.25	0.25	2	0.5	0.5	16	0.12	8	16	16	2	0.5	10
W43	≤0.5	≤4	≤8	0.5	1	0.12	0.25	0.25	0.25	1	0.5	0.5	1	0.12	8	16	0.5	2	0.5	10
W52	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	16	0.12	8	32	8	2	0.5	10
W53	≤0.5	≤4	≤8	16	16	8	2	8	8	1	0.5	2	16	0.12	8	16	32	8	0.5	320
W56	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	1	0.12	8	32	8	2	0.5	10
W59	≤0.5	≤4	≤8	8	8	4	1	1	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	320
W60	≤0.5	≤4	≤8	16	16	4	1	8	8	1	0.5	1	16	0.12	8	16	32	2	0.5	320
W61	≤0.5	≤4	≤8	4	2	4	2	0.25	0.25	2	0.5	0.5	1	0.12	8	32	0.5	2	0.5	80
W63	≤0.5	≤4	≤8	0.5	1	0.25	0.25	8	0.25	2	0.5	0.5	1	0.12	8	16	8	2	0.5	10
W64	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	16	0.12	8	32	8	2	0.5	10
W65	≤0.5	≤4	≤8	0.5	1	4	2	0.25	0.25	2	0.5	0.5	1	0.12	8	32	0.5	2	0.5	320
W66	≤0.5	≤4	≤8	0.5	16	2	2	0.25	1	2	0.5	1	2	0.12	128	16	32	8	1	320
W69	≤0.5	≤4	≤8	1	2	4	1	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	10
W70	≤0.5	≤4	≤8	16	16	8	1	8	8	1	0.5	1	16	0.12	8	16	32	8	0.5	320
W75	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	1	0.12	8	32	0.5	2	0.5	10
W88	≤0.5	≤4	≤8	0.5	1	0.12	0.25	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	10
W90	≤0.5	≤4	≤8	0.5	1	0.12	0.25	0.25	0.25	2	0.5	0.5	16	0.12	8	16	8	2	0.5	10
W91	≤0.5	≤4	≤8	0.5	4	0.25	0.25	0.25	0.25	2	1	1	16	0.12	8	16	16	2	0.5	10
W95	≤0.5	≤4	≤8	0.5	0.5	0.25	0.25	0.25	0.25	2	0.5	0.5	1	0.12	8	32	16	2	0.5	10
W96	≤0.5	≤4	≤8	0.5	1	0.25	0.25	0.25	0.25	2	0.5	1	1	0.12	8	16	16	2	0.5	10
W103	≤0.5	≤4	≤8	4	2	4	1	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	10
W104	≤0.5	≤4	≤8	4	2	4	2	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	20
W105	≤0.5	≤4	≤8	0.5	1	0.12	0.25	0.25	0.25	2	0.5	1	1	0.12	8	16	8	2	0.5	10
W112	≤0.5	≤4	≤8	0.5	1	0.12	0.25	0.25	0.25	2	0.5	0.5	1	0.12	8	16	0.5	2	0.5	10

*Penicillin P, oxacillin OX, cefoxitin FOX, gentamicin GM, tobramycin TOB, levofloxacin LVX, moxifloxacin MXF, erythromycin E, clindamycin DA, linezolid LNZ, teicoplanin TEC, vancomycin VA, tetracycline TE, tigecycline TGC, fosfomycin FOS, nitrofurantoin NIT, fusidic Acid FA, mupirocin MUP, rifampicin RA and sulfamethoxazole/trimethoprim SXT.

Table 2: The MIC values of MRSA isolates from wound swabs

Isolates		Antimicrobial agents																		
	P	OX	FOX	GM	TOB	LVX	MXF	E	DA	LNZ	TEC	VA	TE	TGC	FOS	NIT	FA	MUP	RA	SXT
W01	R	R	R	R	R	R	I	R	R	S	S	S	R	S	S	S	R		S	R
W02	R	R	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
W06	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
W08	R	R	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
W10	R	R	R	I	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R
W19	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	S
W21	R	R	R	I	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R
W27	R	R	R	S	R	R	R	R	R	S	S	S	S	S	S	S	I	S	S	S
W34	R	R	R	R	I	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S
W39	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S

Isolates		Antimicrobial agents																		
	P	OX	FOX	GM	TOB	LVX	MXF	E	DA	LNZ	TEC	VA	TE	TGC	FOS	NIT	FA	MUP	RA	SXT
W43	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
W52	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S
W53	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	R		S	R
W56	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
W59	R	R	R	I	I	R	I	I	S	S	S	S	S	S	S	S	S	S	S	R
W60	R	R	R	R	R	R	I	R	R	S	S	S	R	S	S	S	R	S	S	R
W61	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R
W63	R	R	R	S	S	S	S	R	R	S	S	S	S	S	S	S	I	S	S	S
W64	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S
W65	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R
W66	R	R	R	S	R	I	R	S	I	S	S	S	S	S	R	R	R		S	R
W69	R	R	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
W70	R	R	R	R	R	R	I	R	R	S	S	S	R	S	S	S	R		S	R
W75	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
W88	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
W90	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	S
W91	R	R	R	S	S	S	S	R	R	S	S	S	R	S	S	S	I	S	S	S
W95	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
W96	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
W103	R	R	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	S
W104	R	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
W105	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
W112	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 3: Antimicrobial susceptibility of MRSA isolates from wound swabs

16S rRNA Analysis and Phylogenetic Tree

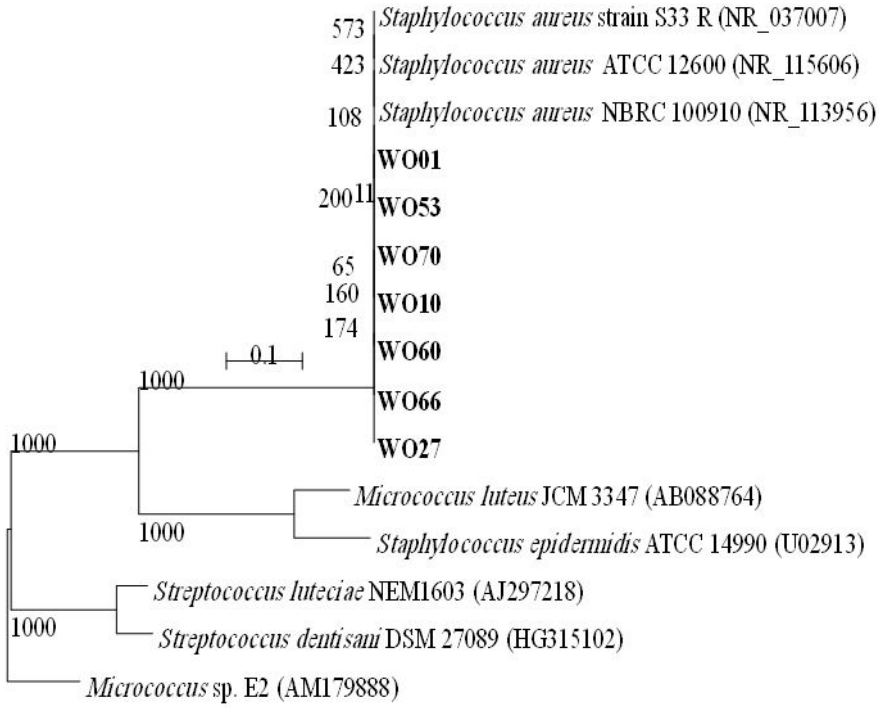

Figure 1: A phylogenetic tree of MRSA isolates from wound swabs based on the nucleotide sequences of 16S rRNA genes was constructed by neighbor-joining method. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates. The GenBank accession numbers of the bacteria are presented in parentheses

For more characterization, 16S rRNA encoding genes of multidrug-resistant (MDR) MRSA isolates WO01, WO10, WO27, WO60, WO66 and WO70 were studied. Initially, the chromosomal DNA was isolated from these isolates. A volume of 5µl of each preparation was resolved by 0.7% agarose gel electrophoresis, confirming the presence of sufficing DNA for PCR reactions. PCR-amplification (~1500 bp) of 16S rRNA encoding genes were achieved and sequenced. The 16S rRNA gene sequences of the MDR-MRSA isolates from wounds were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers: LC107794 (*Staphylococcus aureus* WO01), LC107795 (*Staphylococcus aureus* WO10), LC107796 (*Staphylococcus aureus* WO27), LC107797 (*Staphylococcus aureus* WO53), LC107798 (*Staphylococcus aureus* WO60), LC107799 (*Staphylococcus aureus* WO66) and LC107800 (*Staphylococcus aureus* WO70). The nucleotide sequences of MDR bacterial isolates were compared to existing sequences in the databases. A dendrogram demonstrating the results of 16S rRNA analysis was shown in Figure 1. Results showed highest matching of isolates WO01, WO10, WO27, WO60, WO66 and WO70 to members of the *Staphylococcus* group. As demonstrated, the 16S rRNA sequences of the Staphylococcus isolates are greatest closely associated to *Staphylococcus aureus*. These results are compatible with the conclusions of the morphological and biochemical characterization. The 16S rRNA gene of isolates WO01, WO10, WO27, WO60, WO66 and WO70 shares 99% similarity with that of *Staphylococcus aureus* ATCC 12600. These results suggest that the isolates (WO01, WO10, WO27, WO60, WO66 and WO70) are new isolates of the bacterium *Staphylococcus aureus*.

Antimicrobial Resistance Plasmids

Plasmids were isolated from the most MDR MRSA isolates WO01, WO10, WO27, WO60, WO66 and WO70 recovered from wounds. A volume of 15µl of each plasmid preparation was analyzed by 0.7% agarose gel electrophoresis. As showing in (Figure 2 and Table 4), all isolates contained 1-3 plasmids. Most isolates carried a plasmid of > 10 kb. The isolates WO01, WO10, WO60, WO66 and WO70 contained a plasmid of > 10 kb. The isolates WO01 and WO60 appeared to be carried 4 plasmids with different sizes (2kb, 4kb, >10kb, >10kb).

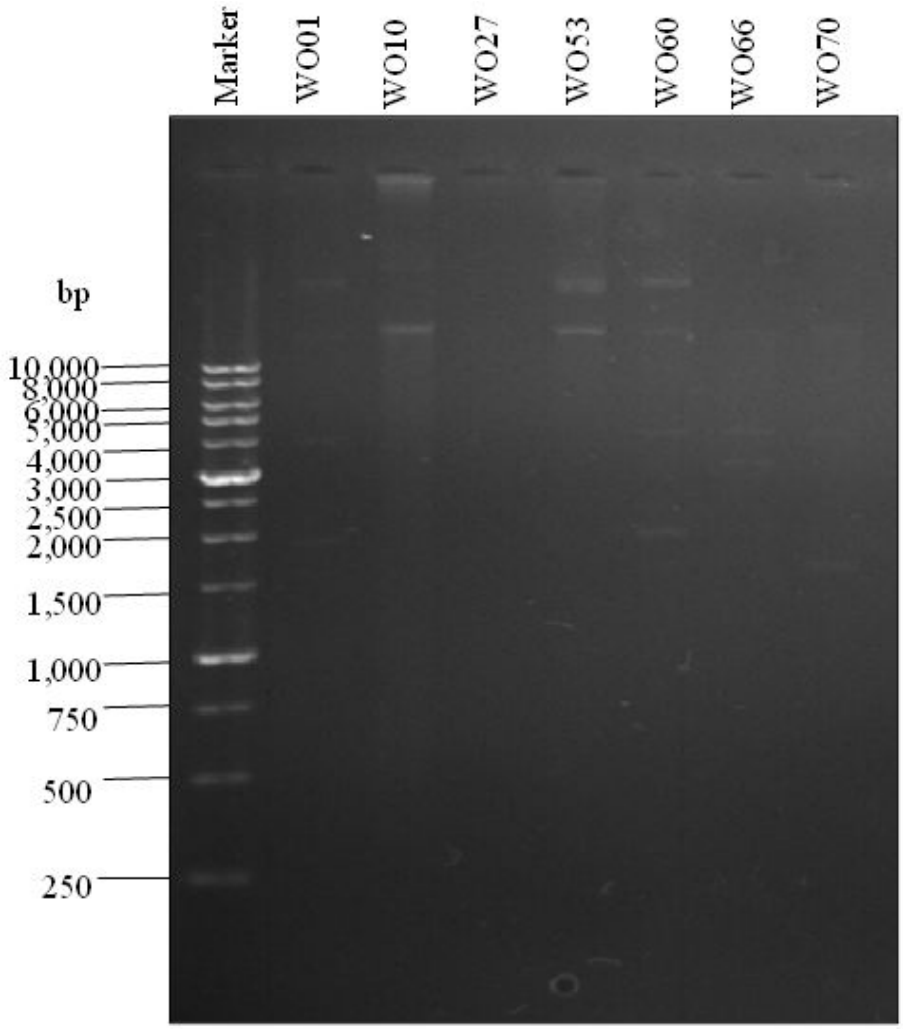

Figure 2: 1% Agarose gel electrophoresis of plasmids isolated from MRSA isolates W01, WO10, WO27, WO53, WO60, WO66 and WO70 obtained from wound swabs

Isolates	plasmids patterns (kb)	Total number
WO01	2kb,4kb, >10kb, >10kb	4
WO10	>10kb	1
WO27	0	0
WO53	>10kb,>10kb.	2
WO60	2kb,4kb, >10kb, >10kb	4
WO66	3kb,4kb,>10kb	3
WO70	>1.5kb,4kb,>10kb	3

Table 4: Plasmid profiles of MRSA isolates from wound swabs

Antimicrobial Resistance Genes

Genes responsible for antimicrobial resistance were investigated in MDR-MRSA isolates (resistant to 5 or more antimicrobial agents) WO01, WO10, WO27, WO53, WO60, WO66 and WO70. The presence of genes responsible for resistance to methicillin (*mecA*), aminoglycoside (*aacA-aphD*), tetracycline (*tetKM*), streptogramin A (*vatABC*) and macrolide-lincosamide-streptogramin B (ermAC) were dtected by PCR (Figures 3 and 4) (Table 5). Also, 16S rDNA and *S. aureus*- specific sequence were determined as amplification control. These genes were PCR amplified of the isolates WO01, WO10, WO27, WO53, WO60, WO66 and WO70. A volume of 5 µl of each PCR reaction was analyzed by 0.7% agarose gel electrophoresis which confirmed the PCR-products were of the expected sizes. These results revealed that the 16S rDNA and *S. aureus*- specific sequence were detected in all isolates. The presence of resistance genes to methicillin (*mecA*) was detected in the isolates WO01, WO10, WO27, WO60, WO66 and WO70 except the isolate WO53. Macrolide-lincosamide-streptogramin B (*ermA*) gene was detected in all isolates. Tetracycline (*tetM*) gene was found in the isolates WO01, WO53, WO60, WO66 and WO70 except isolates WO10 and WO27.The genes responsible for tetracycline (*tetK*), streptogramin A (*vatABC*) and macrolide-lincosamide-streptogramin B (*ermC*) were not detected in all samples. In this study, mecA gene was detected in 86% MRSA isolates.

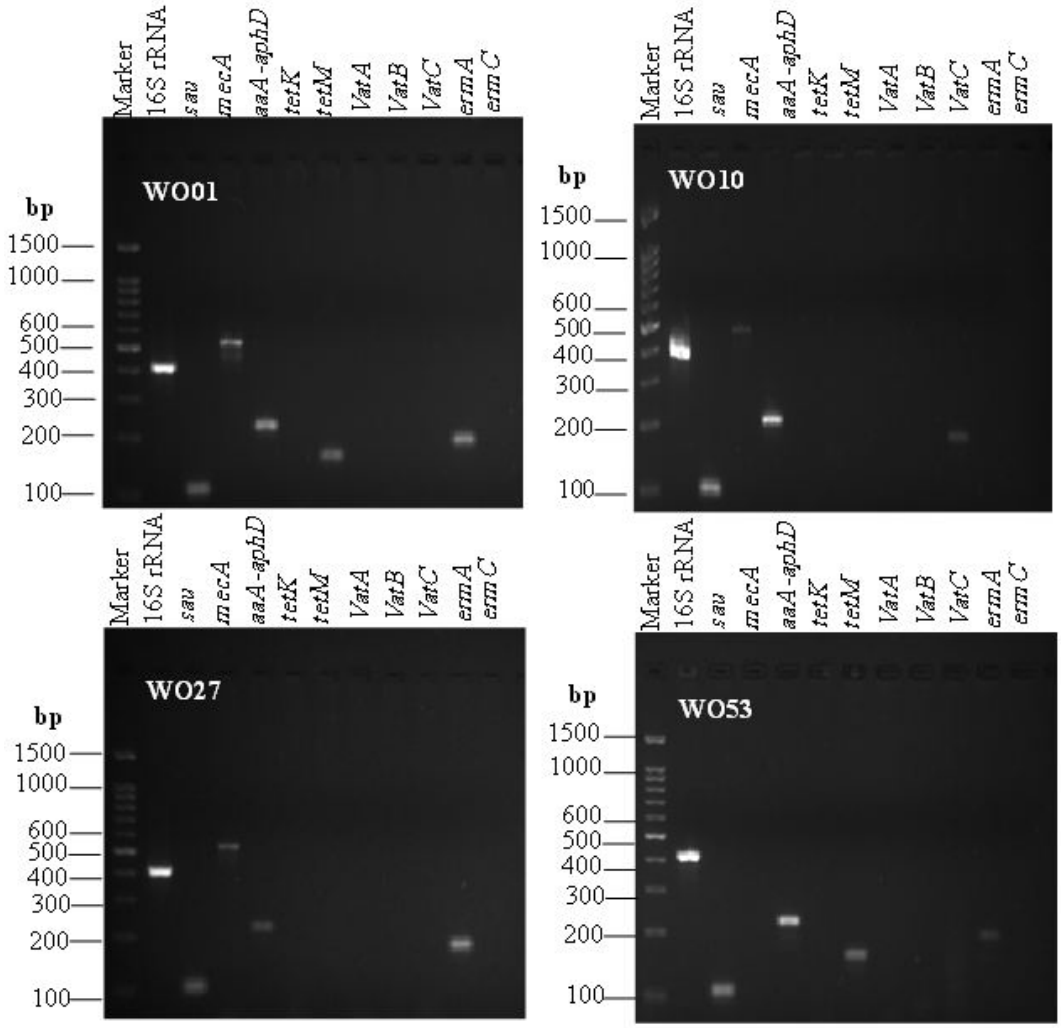

Figure 3: 1% agarose gel electrophoresis of PCR amplified resistance genes of isolates WO01, WO10, WO27 and WO53

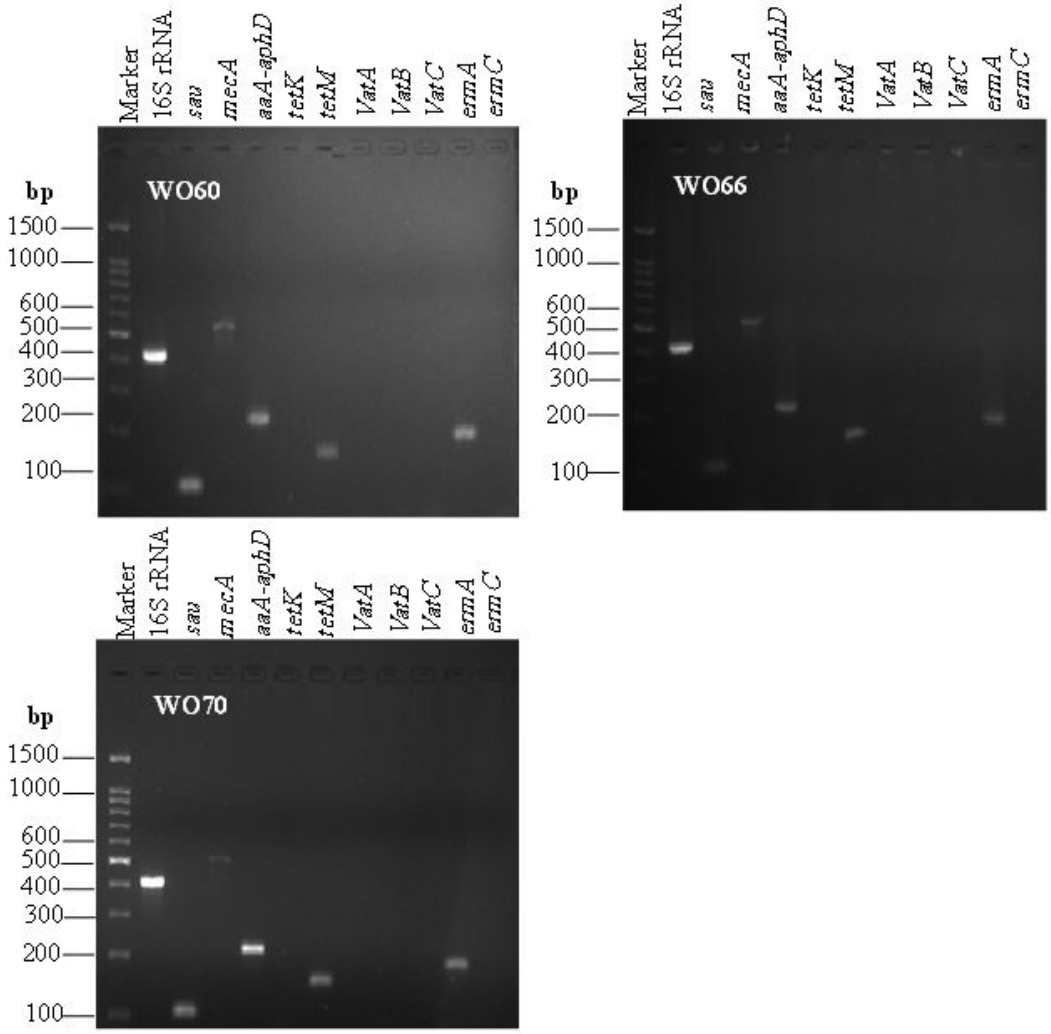

Figure 4: 1% agarose gel electrophoresis of PCR amplified resistance genes of isolates WO60, WO66 and WO70

Isolates	Resistance gens									
	Sau	*mecA*	*aaA-aphD*	*tetK*	*tetM*	*VatA*	*VatB*	*VatC*	*ermA*	*ermC*
WO01	+	+	+	-	+	-	-	-	+	-
WO10	+	+	+	-	_	-	-	-	+	-
WO27	+	+	+	-	_	-	-	-	+	-
WO53	+	-	+	-	+	-	-	-	+	-
WO60	+	+	+	-	+	-	-	-	+	-
WO66	+	+	+	-	+	-	-	-	+	-
WO70	+	+	+	-	+	-	-	-	+	-

+, positive; -, negative

Table 5: The rates of antibiotic resistance genes in MRSA isolates from wound swabs

Discussion

Of the 67 *Staphylococcus aureus* isolates from wound specimens, 33 (49%) were positive for MRSA. *S. aureus* is able to coagulate extracellular plasma, and can thus be distinguished from most other staphylococci as described by Bannerman [13]. In the present study, the prevalence of MSSA *S. aureus* and MRSA wound carriage among patients was 51 and 49 %, respectively. Previous study demonstrated that of the 1,353 *S. aureus*-positive encounters recorded, 431 (32%) grew MRSA and 922 (68%) wounds grew methicillin-susceptible *S. aureus* [14].

All MRSA isolates were resistant to penicillin, oxacillin and cefoxitin. However, 100% of MRSA isolates were determined as sensitive to linezolid, teicoplanin, vancomycin, tigecycline, mupirocin and rifampicin. Earlier results reported that 75%, 51.4% and 73.8% from wounds, skin, and bed, respectively, were found to be methicillin-resistant *Staphylococcus aureus* (MRSA) using the disc-sensitive test methods. The antimicrobial susceptibility pattern results showed the level of resistance to be ampicillin 100% in all the

three sites, pefloxacin 90.9%, 72.2%, 66.7%, ceftriaxone 69.7%, 72.2%, 70.8%, gentamicin 54.5%, 52.8%, 37.5%, and ciprofloxacin 51.5%, 47.2%, 35.4% at the wound, skin, and bed sites, respectively [15,16]. These results confirmed that MRSA continues to pose a threat to the hospitalized patients, especially those with bone and wound infections. The prevalence of MRSA was found to be 49% in the present study which is lower than that with other reports. MRSA showed high resistance (100%) to penicillin and cefoxitin followed by erythromycin (99%). However, MRSA exhibited 100% susceptibility to vancomycin and linezolid. They had also found 7 vancomycin intermediate sensitive *S. aureus* (VISA) isolates [17]. It is concluded that MRSA is potential threat to public health. All MRSA isolates from different clinical samples were susceptible to linezolid, vancomycin, and teicoplanin [18]. Vancomycin and linezolid could be prescribed as a drug of choice in treating MRSA associated infections.

In the present study, 78% of MRSA isolates were MDR. The multi-drug resistance in MRSA isolates has been a major problem worldwide which leads to ineffective therapy and increase in treatment expenses [19]. The present study highlighted that high number of MRSA were multi-drug resistant which is in line with the previous findings which reported high rate of multi-drug resistance in MRSA [20]. We have found that all MRSA isolates tested in the present study were fully sensitive to vancomycin so it may be used as the drug of choice for treating multidrug-resistant MRSA infections. Similar findings were reported by various previous studies [21]. In addition, all isolates were sensitive to linezolid which is in line with other studies [22]. All MRSA isolates were resistant to cefoxitin which is in line with other studies [23]. MRSA resistance to quinolones (ciprofloxacin) was also found to be very high (80.19%) in the current study. This correlates with an earlier finding where it has been shown that MRSA was 86.59% resistant to ciprofloxacin [24].

Plasmid profile has been reported as one of the techniques for typing MRSA and MSSA [25]. In this study, 86% MRSA isolates had plasmids. Plasmid profile analysis appears to be of very low discriminatory capacity in the investigation of MRSA epidemiology because of the non-detection of plasmids in 14% of isolates. Plasmid analysis of representative *S. aureus* isolates demonstrates the presence of a wide range of plasmid sizes, with consistent rela¬tionship between plasmid profiles and resistance phenotypes. Previous study reported that the 19 non-multidrug-resistant isolates (NMR) that did not harbour plasmids were only resistant to methicillin whereas all the NMR isolates harbouring at least one plasmid were resistant to at least one additional antibiotic. We conclude that although plasmid carriage plays an important role in antibiotic resistance, especially in NMR-HA-MRSA and CA-MRSA, the multidrug resistance phenotype from HA-MRSA is not associated with increased plasmid carriage and indeed is characterized by an absence of plasmid DNA [26].

The presence of resistance genes to methicillin (*mecA*) was detected in all MDR-MRSA except the isolate WO53. Macrolide-lincosamide-streptogramin B (*ermA*) gene was detected in all isolates. Tetracycline (*tetM*) gene was found in all MDR-MRSA except isolates WO10 and WO27.The genes responsible for tetracycline (*tetK*), streptogramin A (vatABC) and macrolide-lincosamide-streptogramin B (*ermC*) were not detected in all samples. In this study, mecA gene was detected in 86% MRSA isolates. Previous results reported that PCR results indicated that 62 (92.53%) out of 67 MRSA isolates were positive for *mecA* whereas 7.46% remaining oxacillin-resistant isolates (which was mecA negative) must be MRSA because of some other mechanisms [27,28]. Previous results reported that the *tetM/K, blaZ, aacA-aphD, ermC* and *mecA* genes were detected in clinical isolates however *ermA* gene was not found [29].

Conclusion

The present study highlighted moderate percentage of MRSA and their multidrug resistance of patients in Taif province. The MDR-MRSA is a potential threat to the public health at Taif province as this bacterium can distribute in community causing severe clinical conditions. Linezolid, teicoplanin, vancomycin, mupirocin and rifampicin could be the drugs of choice in treating MRSA infection. Moreover, MIC of vancomycin should be performed routinely to screen out vancomycin-intermediate *Staphylococcus aureus* (VISA). Extensive studies are needed in different geographical areas of the Taif province to better understand the epidemiology and molecular mechanisms of drug resistance in MRSA.

References

1. Deleo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated meticillin-resistant Staphylococcus aureus. Lancet 375: 1557-68.

2. Chambers HF, DeLeo FR (2009) Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol 7: 629-41.

3. Perwaiz S, Barakzi Q, Farooqi BJ, Khursheed N, Sabir N (2007) Antimicrobial susceptibility pattern of clinical isolates of methicillin-resistant Staphylococcus aureus. J Pak Med Assoc 57: 2-4.

4. Malachowa N, DeLeo FR (2010) Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci . 67: 3057-71.

5. van Den Braak N, van Belkum A, Kreft D, te Witt R, Verbrugh HA, et al. (1999) The prevalence and clonal expansion of high-level gentamicin-resistant enterococci isolated from blood cultures in a Dutch university hospital. J Antimicrob Chemother 44: 795-8.

6. Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. J Clin Microbiol 41: 4089-94.

7. Michalova E, Novotna P, Schlegelova J (2004) Tetracyclines in veterinary medicine and bacterial resistance to them. Vet Med Czech 49: 79-100.

8. World Health Organization (2016) Antibiotic resistance.

9. Van Enk RA, Thompson KD (1992) Use of a primary isolation medium for recovery of methicillin-resistant Staphylococcus aureus J Clin. Microbiol 30: 504-5.

10. Brenner DJ, Krieg NR, Staley JT, Garrity GM (2005) Bergey's Manual of Systematic Bacteriology, (2nd edn), Switzerland.

11. Mahon C, Lehman D, Manuselis G (2011) Textbook of diagnostic microbiology (4 edn) Maryland Heights: Saunders Elsevier.

12. CLSI (2015) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute, USA.

13. Bannerman TL, Murray PR, Baron EJ, Jorgensen JM (2003) Staphylococci, Micrococcus and other catalase-positive cocci that grow aerobically. American Society for Microbiology Press, USA 2003: 384- 404.

14. Borgundvaag B, Ng W, Rowe B, Katz K (2013) Prevalence of methicillin-resistant Staphylococcus aureus in skin and soft tissue infections in patients presenting to Canadian emergency departments. CJEM 15:141-60.

15. Udobi CE, Obajuluwa AF, Onaolapo JA (2013) Prevalence and antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus from an orthopaedic hospital in Nigeria. Biomed Res Int 2013: 860467.

16. Tadesse S, Alemayehu H, Tenna A, Tadesse G, Tessema TS, et al. (2018) Antimicrobial resistance profile of Staphylococcus aureus isolated from patients with infection at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. BMC Pharmacol Toxicol 19: 24.

17. Ullah A, Qasim M, Rahman H, Khan J, Haroon M, et al. (2016) High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. SpringerPlus 5: 600.

18. Udo EE, Boswihi SS (2017) Antibiotic resistance trends in methicillin-resistant Staphylococcus aureus isolated in Kuwait Hospitals: 2011–2015. Med Princ Pract 26: 485-90.

19. Kock R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, et al. (2010) Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill 15: 1-9.

20. Khan AB, Ahmed F, Cha SB J, Shin M, Bashir S, et al. (2015) Antibiotic resistance pattern and molecular epidemiology of methicillin-resistant Staphylococcus aureus colonization in burns unit of a tertiary care hospital in Peshawar, Pakistan. Trop J Pharm Res 13: 2091-9.

21. Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN (2014) Isolation of MRSA, ESBL and AmpC–β-lactamases from neonatal sepsis at a tertiary care hospital. J Clin Diagn Res 8: DC24.

22. Vijayamohan N, Nair SP (2014) A study of the prevalence of methicillin resistant Staphylococcus aureus in dermatology inpatients. Indian Dermatol Online J 5: 441-5.

23. Ahmad B, Khan F, Ahmed J, Cha SB, Shin M, et al. (2015) Antibiotic resistance pattern and molecular epidemiology of methicillin-resistant Staphylococcus aureus colonization in burns unit of a tertiary care hospital in Peshawar, Pakistan. Trop J Pharm Res13: 2091-9.

24. Pandya N, Chaudhary A, Mehta S, Parmar R (2014) Characterization of methicillin resistant Staphylococcus aureus from various clinical samples at tertiary care hospital of rural Gujarat. J Res Med Dent Sci 5: 202-6

25. Tayfour MA, Eris FN, Alanazi AR (2005) Comparison of antibiotic suscep¬tibility tests, plasmid profiles and restriction enzyme analysis of plasmid DNA of methicillin susceptible and resistant-Staphylo¬coccus aureus strains isolated from intensive care units. Saudi Med J 2: 57-63.

26. Caddick JM, Hilton AC, Rollason J, Lambert PA, Worthington T, et al. (2005) Molecular analysis of methicillin-resistant Staphylococcus aureus reveals an absence of plasmid DNA in multidrug-resistant isolates. FEMS Immunol Med Microbiol 44: 297-302.

27. Shahkarami F, Rashki A, Ghalehnoo ZR (2014) Microbial susceptibility and plasmid profiles of methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus. Jundishapur J Microbiol 7: e16984.

28. Chambers HF (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev10: 781-91.

29. Achek R, Hotzel H, Cantekin Z, Ibrahim Nabi, Hamdi TM, et al. (2018) Emerging of antimicrobial resistance in staphylococci isolated from clinical and food samples in Algeria. BMC Res Notes 11: 663.