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Research Article
Molecular Characterization of *mec*A and SCCmec Genes in Pathogenic *Staphylococcus* spp. Collected from Hospitals in Taif Region, KSA

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Abstract
Background and Objective: Staphylococcus is a group of bacteria that can cause a multitude of diseases. Bacteria isolates were collected from different hospitals in Taif governorate in KSA. Out of 89 isolates, eight isolates of *Staphylococcus* spp. were subjected to fingerprinting based on their resistance/susceptibility to antibiotics which are commonly used in KSA and all over the world. Most of the isolates showed multidrug resistance. Interestingly, out of the eight isolate, Staphylococcus isolate number 6 found to be as Methicillin Resistant *Staphylococcus epidermidis* (MRSE). **Methodology:** It was identified using the sequencing of 16S rDNA. Methicillin resistance in Staphylococcus isolates is primarily mediated by the *mec*A gene, which codes for the modified penicillin-binding protein 2a, *mec*A is carried on a mobile genetic element that called the Staphylococcal Cassette Chromosome mec (SCCmec). Thus in the present study, the different genes of *mec*A and SCCmec were verified in the eight isolates using primer specific PCR technique. The *mec*A I gene (with size about 162 bp long) and *mec*A III gene (with size about 449 bp long) were found in the eight isolates. Interestingly, *mec*A II gene (with size about of 540 bp long) was observed in only five isolates. Surprisingly, SCCmec I gene was not found in any of the eight isolates. While, SCCmec II and SCCmec III gene were observed in only six isolates. The repetitive element PCR technique (Rep-PCR) was used to study the genetic similarity between the examined isolates. Six primers were used in this study. **Results:** The results revealed that approximately 108 different banding patterns were obtained, dividing the isolates into two clusters. **Conclusion:** Results refer to the PCR assay offers a rapid, simple and accurate identification of antibiotic resistance profiles and could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic resistance determinants in epidemiological studies.

Key words: Rep-PCR, *mec*A, SCCmec genes, antibiotic resistance, *Staphylococcus* spp.

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have been used in clinical practice for about 80 years and throughout that period the problems posed by resistant bacteria have escalated at a pace that has forced near continuous development of new antibacterial drugs. A large variety of antibiotics are currently being used in human and veterinary medicine but their efficacy has been threatened by microbial resistance[1-3]. *Staphylococcus epidermidis* is an emerging nosocomial pathogen, In particular biofilm-forming strains of *S. epidermidis* that are also methicillin-resistant (MRSE) have become a very serious clinical problem[4]. The prevalence of MRSE has increased in many parts of the world causing serious infections in hospitals that pose a serious burden in terms of medical and socioeconomic costs and cause significant morbidity and mortality[5,6]. The resistance in MRSE is due to the expression of Penicillin Binding Protein 2a (PBP2a) that encoded by *mec*A genes[7,8]. The *mec*A genes are located on the mobile genetic elements that called Staphylococcal Cassette Chromosome (SCC)[9,10]. The SCC is a large genetic mobile element which differs in size and genetic composition among different strains of MRSE[5,6,11]. Different types of SCCmec cassettes were extensively studied by PCR techniques[9,12]. In addition, other antibiotic resistance genes may also be present in the cassette rendering resistance to multiple antibiotics[13,14]. Detection of *mec*A gene by PCR is considered as the gold standard[15,16]. For methicillin resistance as these genes are highly conserved among staphylococcal species.

In the present study, the PCR method was used for the detection of *mec*A genes among the Staphylococcus isolates additionally, various classes of repeated DNA sequences also have been used in this study, which described in diverse prokaryotic genomes[14,17], as a tool for genetic sub typing of the different Staphylococcus isolates. Repetitive element sequence-based PCR (rep-PCR) is a new typing method that differentiates microbes by using primers complementary to interspersed repetitive consensus sequences that enable amplification of diverse-sized DNA fragments consisting of sequences between the repetitive elements[18,19]. Multiple amplicons of different sizes can be fractioned by electrophoresis and the resulting DNA fingerprint patterns, specific for individual bacterial clones, can be compared. Numerous studies have shown that the application of rep-PCR using oligonucleotide primers based on the repetitive extragenic palindromic (Rep) elements (Rep-PCR)[20] or on the Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences (ERIC-PCR) has been successful in typing a variety of bacteria[20,21].

MATERIALS AND METHODS

Sample collection and growth: About 89 clinical samples of urine and stool were collected from hospitalized patients in the Taif hospitals, KSA, about one year period. The swabs of urine and stools samples were taken in aseptic conditions and were transported immediately to the microbial genetics laboratory at Deanship of Scientific Research, Taif University, KSA and processed for direct Gram staining followed by culture on blood agar, mannitol, salt agar and Mac Conkey agar (Himedia) and were incubated at 37°C for 24 h. The β-hemolytic colonies on the blood agar plate, the yellow colored colonies on the mannitol salt agar and pink colored colonies on Mac Conkey agar were subjected for Gram staining as previously reported[22]. The organisms identified as *S. aureus* were tested further for β-lactamase production by *mec*A genes.

Isolation and purification of clinical bacterial isolates: Sterile dry swabs were used for streaking of clinical samples onto sterile petri dishes containing nutrient agar media (Biolife, USA). Inoculated streaked dishes were incubated at 28°C for 48 h. Single colony was picked up by sterile inoculation needles and then sloped into cultures of nutrient agar media.

Antibiotic sensitivity test: About nine types of antibiotics, 1 µg oxacillin (OX), 10 µg gentamicin (GM), 5 µg vancomycin (VA), 30 mg amoxicillin (AMC), 30 µg cefotaxime (CTX), 30 µg amikacin (AN), 30 µg cefazolin (Cz), 15 µg erthromycin (E) and 30 µg tetracycline (Te) were used for disc diffusion bioassay[23]. Clinical bacterial isolates suspensions were spread by sterile glass rods on the surface of nutrient agar media. Then antibiotic discs (Bioanalyse®) were placed onto the surface of the inoculated nutrient agar plates. The plates were then incubated at 28°C for 48 h and then inhibition zones were observed and measured in mm.

Biochemical characteristics of antibiotic resistant bacteria: The cultural, morphological and biochemical criteria of the eight Staphylococcus antibiotic resistant bacteria isolates were used as a taxonomic criteria. Gram stain was the key step and was carried out as previously reported[10].

Genomic DNA extraction: The cell pellets from all isolates were used to extract genomic DNA using extraction kit (Jena Bioscience, Germany) following the manufacturer's instructions.

Rep-PCR technique: For repetitive sequence analysis, PCR conditions for Staphylococcus isolates in the present investigations were standardized. Seven repetitive sequence primers were used to amplify genomic DNA of the eight isolates according to Hassan *et al.*[24]. Out of these, six primers which presented strong band resolution were chosen for the study, BOX A1(5'-CTA CGG CAA GGC GAC GCT GAC G-3'), REP1R-I (5'-III ICG ICG ICA TCI GGC-3') (forward), REP2-I (5'-ICG ICT TAT CIG GCC TAC-3') (reverse), MBO-REP1 (5'-CCG CCG TTG CCG CCG TTG CCG CCG-3'), (GTG)5 (5'-GTG GTG GTGGTG GTG-3'), REP2-II (5'-GAGAGAGAGAGAGAGAA-3') and REP8-I (5'-GAC GAGAGAGAGAGAGAGA-3'). Following the experiments for optimization of component concentrations, PCR amplification of repetitive sequence primers were carried out in 25 µL volume containing 1 µL (about 20 ng) of genomic DNA, 12.5 µL of Go Taq® Green Master Mix (Promega, USA), 1 µL of primer (20 pmol) and deionized distilled water (up to a total volume of 25 µL). For DNA amplification, the C1000TM Thermo Cycler Bio-Rad, Germany, was programmed under the conditions involving denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, primer annealing at 52°C for 45 sec and primer extension at 72°C for 2.5 min, final extension step at 72°C for 10 min.

Detection of antibiotic resistance genes: The PCR amplification for detection of the antibiotic genes *mec*A and SCCmec was carried out as previously reported[25,26] using specific primers that showing in Table 1. The PCR reactions were carried out in a total volume of 25 µL containing 1X PCR buffer, dNTPs, 1 units of *Taq* DNA polymerases, 10 pmol of each primer and the DNA template. The PCR amplifications were performed in C1000TM Thermo Cycler (Bio-Rad). The PCR conditions for *mec*A genes was: 95°C for 5 min, followed by 30 repeated cycles of 30 sec at 94°C, 30 sec of annealing at 54°C and 1 min of extension at 72°C, followed by 7 min as final extension at 72°C. The PCR conditions for SCCmec genes were: 95°C for 5 min, 30 cycles of denaturation at 95°C, annealing at 58°C and extension at 72°C for 1 min each, followed by a final extension of 10 min at 72°C. Amplification products (10 µL) were analyzed on 1.5% agarose gels stained with ethidium bromide and visualized by UV illumination and were photographed by a Bio-Rad Gel Doc 2000 device.

Table 1: Primers name and sequences of antibiotic resistance genes of Staphylococcus

Primers name	Primers sequence 5'------3'	Amplicon size (pb)
*mec*A I-F	TCC AGA TTA CAA CTT CAC CAG G	162
*mec*A I-R	CCACTTC ATA TCT TGT AAC G	
*mec*A II-F	ATC GAT GGT AAA GGT TGG C	540
*mec*A II-R	AGT TCT GCA GTA CCG GAT TTG C	
*mec*A III-F	CTC AGG TAC TGC TAT CCA CC	449
*mec*A III-R	CAC TTG GTA TAT CTT CAC C	
SCCmec I-F	GCT TTA AAG AGT GTC GTT ACA GG	613
SCCmec I-R	GTT CTC TCA TAG TAT GAC GTC C	
SCCmec II-F	CGT TGA AGA TGA TGA AGC G	398
SCCmec II-R	CGA AAT CAA TGG TTA ATG GAC C	
SCCmec III-F	CCA TAT TGT GTA CGA TGC G	280
SCCmec III-R	CCT TAG TTG TCG TAA CAG ATC G	

PCR amplification of 16S-rRNA gene: The DNA (2 µL), approximately 50 ng, was used as template for the polymerase chain reaction assays. The amplifications of 16S rDNA gene amplification were done according to Hassan and Ismail[27].

Sequencing of 16S-rRNA gene: About 900 bp 16S rDNA fragments were purified using QIA quick PCR purification kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions and sequenced with the same primers using the sequencer (Gene analyzer 3121). The bacterial 16S-rDNA sequences obtained were then aligned with known 16S-rDNA sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information and percent homology scores were generated to identify bacteria.

Data analysis: In order to determine the genetic relationship among studied bacteria, Rep-PCR data were scored for presence (1) or absence (0) of the bands using Gene Tools software from Syngene. A simple matching coefficient was estimated by means of the Jaccard's coefficient to construct a similarity matrix. Cluster analysis and dendrogram were produced on the basis of the unweighted average pair group method (UPGMA) using the NTSYS-PC Statistical Package[28].

RESULTS AND DISCUSSION

Isolation of antibiotic resistant bacteria: About 89 clinical samples of urine and stool swabs were collected from Saudi Arabian patients in Taif city and were then analyzed for presence of pathogenic bacteria on nutrient agar media. All samples showed obvious bacterial cultures that were surveyed for presence of multidrug resistant bacteria. Out of 89 cultures tested, 8 multidrug resistant bacteria isolates were identified as *Staphylococcus* spp. and selected for isolating the antibiotic resistance genes.

Biochemical characteristics of multidrug resistant bacteria: Based on molecular, cultural, morphological and biochemical characteristics, eight isolates were belonging to *Staphylococcus* spp. and they were characterized as Gram positive, short coccui bacteria and non-motile cells. Other

biochemical tests showed negative results. Similar results were obtained by El-Amin and Faidah[29]. They identified as Staphylococcus strains that isolated from hospitals of western region in Saudi Arabia based on Gram stain, catalase and coagulase tests[29].

Antibiotic susceptibility testing: Since the introduction of antibiotics in medicine, Staphylococcus strains has shown a frequent and rapid development and spread of antibiotic resistance and has developed resistance to all types of antibiotics such as tetracyclines, which are broad-spectrum antibiotics used in the treatment and prevention of bacterial infections[23,27,30]. Most tetracycline resistant bacteria have acquired tetracycline resistance genes[8]. In the present study, antimicrobial susceptibility testing was performed by the Bioanalyse Disc Diffusion method using nutrient agar plates, according to the Clinical and Laboratory Standards Institute guidelines[31]. The antimicrobial agents tested and their corresponding concentrations were described previously in the material and methods section. After incubating the inoculated plates aerobically at 28°C for 48 h, the susceptibility of the eight Staphylococcus isolates to each antimicrobial agent was detected using Combination Disk Diffusion Test[32]. The results were interpreted in accordance with criteria provided by CLSI[23,31]. Most of all of the eight Staphylococcus isolates were resistant to one or more antimicrobial agent. Interestingly, the Staphylococcus 6 isolate showed resistance to all antibiotics agents tested in this study. This isolate was characterized and identified by sequence analysis of 16S rDNA region. We found it belonging to *Staphylococcus epidermidis* with highly genetic similarity to *Staphylococcus epidermidis* strain AU12-03. On the other hand, Staphylococcus 5 isolate was sensitive to all tested antibiotics (Table 2 and Fig. 1). Seven of the Staphylococcus isolates were found to be sensitive to the gentamicin, cefotaxime, amikacin and cefazolin (Table 2). Only two isolates were found to be sensitive to vancomycin, erthromycin and tetracycline (Table 2). Additionally, seven of the Staphylococcus isolates were found to be resistant to oxacillin (Table 2). It was clearly reported that methicillin resistance in staphylococci is mainly due to the expression of the *mec*-gene[23,31]. Methicillin Resistant *Staphylococcus epidermidis* (MRSE) is increasingly important nosocomial pathogen particularly in a Neonatal Intensive Care Unit (NICU)[5,8]. The high risk factors in new-borns, in addition to the context of intensive care, very low birth weight, prematurity, immaturity of immunological defences, impaired ability to opsonize and kill staphylococci[5,10].

Table 2: Antimicrobial resistance profiles of eight Staphylococcus isolates

Strains	OX	GM	VA	AMC	CTX	AN	CZ	E	TE
Staphylococcus 1	0.0	2.2	1.6	2.0	1.4	2.2	2.0	1.5	3.0
Staphylococcus 2	0.0	1.9	0.0	1.2	4.2	2.0	3.0	0.0	0.0
Staphylococcus 3	0.0	1.6	0.0	1.4	2.4	1.0	2.0	0.0	0.0
Staphylococcus 4	0.0	1.1	0.0	1.1	2.2	1.2	2.1	0.0	0.0
Staphylococcus 5	1.9	1.8	1.3	2.4	3.0	1.4	2.5	2.0	2.2
Staphylococcus 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Staphylococcus 7	0.0	1.6	0.0	1.9	3.1	1.8	3.0	0.0	0.0
Staphylococcus 8	0.0	1.1	0.0	1.4	2.5	1.4	1.5	0.0	0.0

Inhibition zones diameter was measured in mm, OX = 1 μg oxacillin, GM =10 μg gentamicin, VA = 5 μg vancomycin, AMC = 30 mg amoxicillin, CTX =30 μg cefotaxime, AN = 30 μg amikacin, Cz = 30 μg cefazolin, E =15 μg erthromycin, TE =30 μg tetracycline and 0.0 = Complete resistance (complete growth)

PCR analysis for *mec*A and SCCmec genes: The PCR amplification products of *mec*A genes producing in Staphylococcus isolates were shown in Fig. 2-4. Results showed that the three genes were identified in some isolates. Eight isolates of Staphylococcus found to be carried both *mec*A I gene and *mec*A III gene with size about of 162 and 449 bp long, respectively (Fig. 2 and 4). These results, in some aspects, are similar to the reports that published

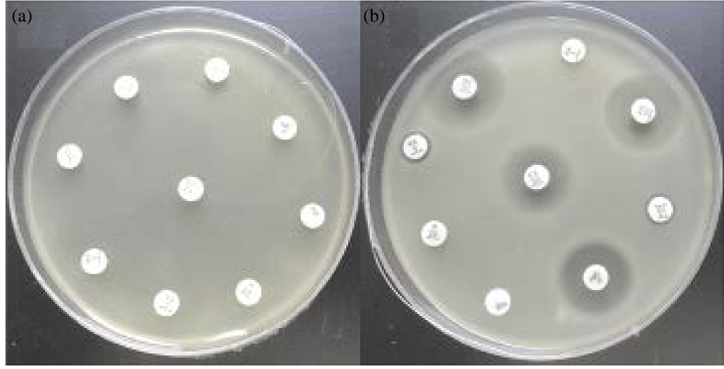

Fig. 1(a-b): Combination disk diffusion test of antibiotic resistance in Staphylococcus isolates number (a) Staphylococcus 6 and (b) Staphylococcus 4

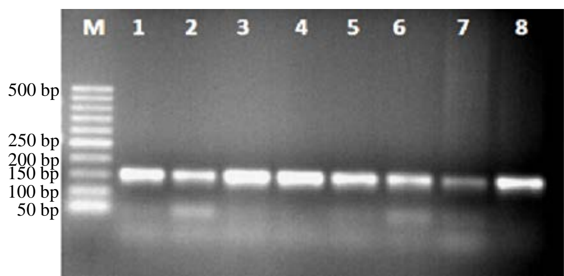

Fig. 2: Amplification of *mec*A I gene producing in Staphylococcus isolates by single PCR with size about of 162 bp. M: 50 bp DNA ladder

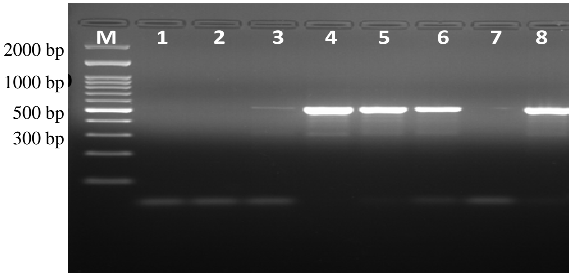

Fig. 3: Amplification of *mec*A II gene producing in Staphylococcus isolates by single PCR with size about of 540 bp

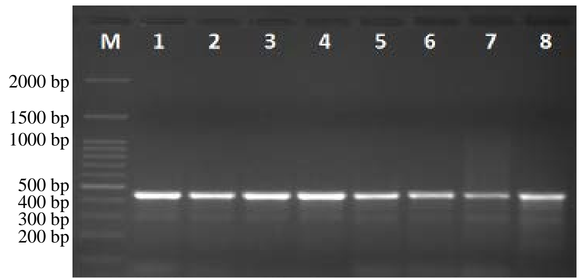

Fig. 4: Amplification of *mec*A III gene producing in Staphylococcus isolates by single PCR with size about of 449 bp

previously in Saudi Arabia[23]. Interestingly, *mec*A II gene, with size about of 540 bp long, was observed only in five isolates (Staphylococcus 3, 4, 5, 6 and 8) (Fig. 3), *mecA* gene is responsible for the resistance of Staphylococcus to the β-lactams antibiotic group. And this genetic material is not belonging to the Staphylococcus genome but it has been acquired by *Staphylococcus* spp. many times over the past 40 years from unknown sources[10]. The gene product of *mec*A is a penicillin binding protein 2a (PBP2a). Staphylococcus normally produces four PBPs enzymes that are anchored on the cytoplasmic membrane[33]. The functions of these enzymes

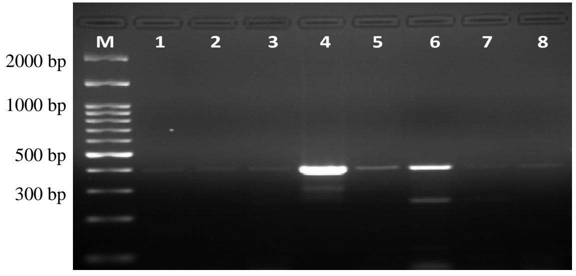

Fig. 5: Amplification of SCCmec II gene producing in Staphylococcus isolates by single PCR with size about of 398 bp

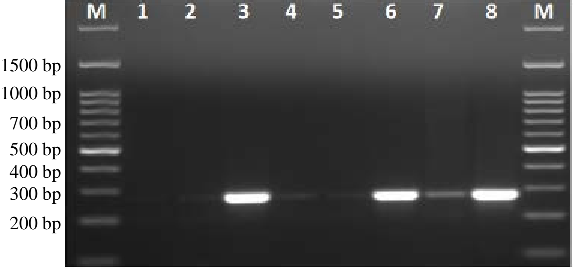

Fig. 6: Amplification of SCCmec III gene producing in Staphylococcus isolates by single PCR with size about of 280 bp

are the assembly and regulation of the latter stages of the cell wall biosynthesis[34]. Whereas these four PBPs are susceptible to modification by β-lactam antibiotics, an event that leads to bacterial death, PBP2a is refractory to the action of all available β-lactam antibiotics. The PBP2a is capable of taking over the functions of the four typical PBPs of Staphylococcus in the face of the challenge by β-lactam antibiotics. Based on the classes of the mec gene complex and the ccr gene types, eleven types (I to XI) of SCCmec have been assigned for Staphylococcus[35]. However, only type I-V is globally distributed while others appear to exist as local strains in the country of origin[35]. Moreover, the PCR ampilicon of SCCmec genes were producing in Staphylococcus isolates. Obviously, only two genes were identified in some isolates of Staphylococcus (Fig. 5 and 6). Surprisingly, SCCmec I gene was not found in any of the eight isolates. The SCCmec II gene with size about of 398 bp long was observed in all eight Staphylococcus isolates (Fig. 5). While, SCCmec III gene (with size about of 280 bp long) was found in only six isolates (Staphylococcus 1, 2, 3, 4, 6 and 8) (Fig. 6). Staphylococcus 6 isolates was observed as highly resistance to all antibiotics tested included methicillin resistant, It have *mec*A and SCCmec genes and molecular identified as *Staphylococcus epidermidis* using 16S rDNA gene so it could identified as Methicillin Resistant

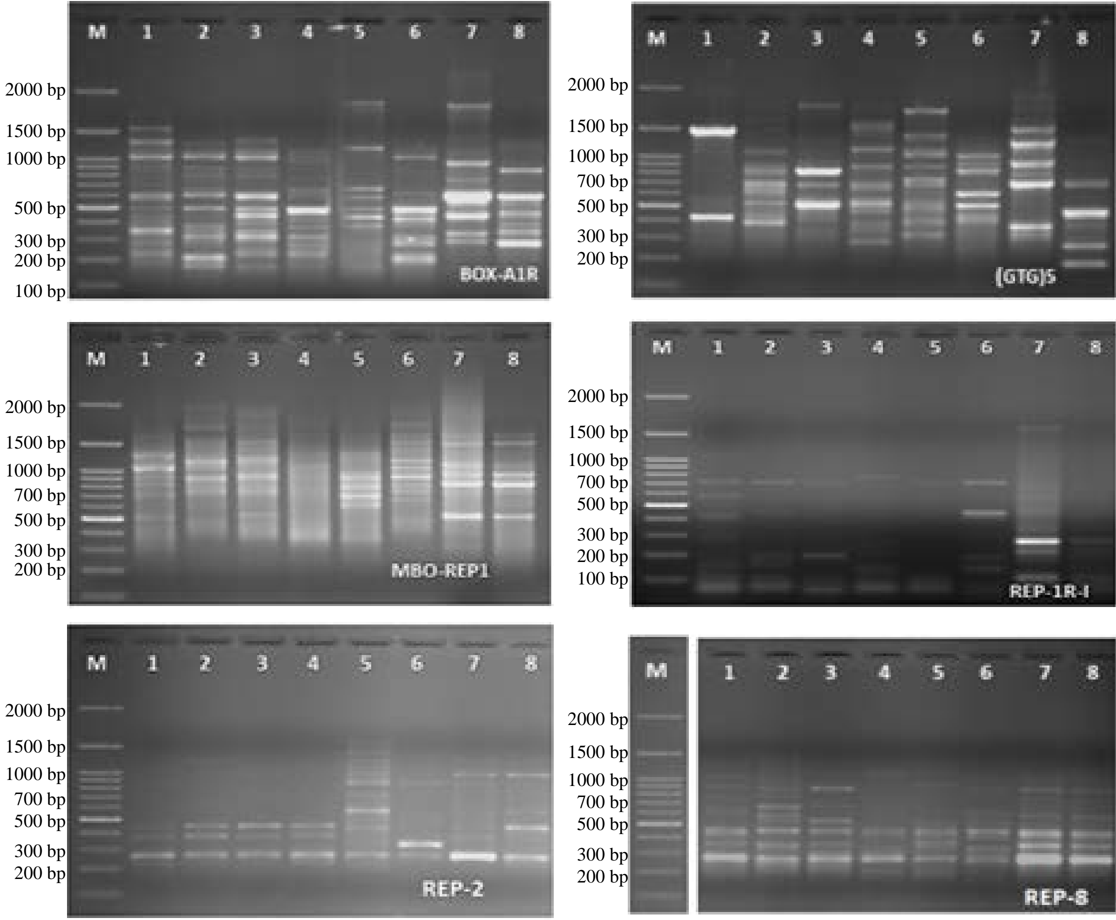

Fig. 7: Rep-PCR profile of eight antibiotic resistance Staphylococcus isolates generated with six repetitive sequence primers, M: 100 bp DNA ladder

Staphylococcus epidermidis (MRSE)[5], who reported that *Staphylococcus epidermidis* is the most common etiologic agent of diseases like bacteremia, osteomyelitis, urinary tract infection and peritonitis caused by ambulatory dialysis with a frequent association with colonization of intravascular catheters and orthopaedic devices. Moreover, methicillin resistant Staphylococcus is resistant to all pencillins, penems, carbapenems and cephalosporis[5].

Rep-PCR analysis: Repetitive element sequence-based PCR (Rep-PCR) is a new typing method that differentiates microbes by using primers complementary to interspersed repetitive consensus sequences that enable amplification of diverse-sized DNA fragments consisting of sequences between the repetitive elements[20]. The Rep-PCR fingerprinting method, utilizing repetitive sequence oligonucleotides, is particularly a powerful tool for genetic studies and it is useful as a screening genotyping method[20]. The Rep-PCR can generate various fingerprint patterns with unlimited number of fragments[24]. In this study, six Rep-PCR primers were used for estimating of genetic diversity of Staphylococcus isolates. The Rep-PCR reactions were performed in duplicate and all amplification products were found to be reproducible (Fig. 7 and Table 3). The Rep-PCR results using primer (BOX A1) has showed a total of 26 bands sized ranged from 150-2230 bp long in all eight Staphylococcus isolates. Five common bands were observed in all isolates which exhibited about 19.2% monomorphism, while the other twenty one fragments have showed 80.8% polymorphism (Table 3). In case of $(GTG)_5$ primer, a total of twenty four fragments have showed 96% polymorphism among the eight Staphylococcus isolates. The molecular size of the amplicon products ranged from 150-2000 bp long. Also, this primer recognized different unique fragments at 150, 200, 400, 1500 and 1800 bp specific to isolate No. 8, 1 and 7, respectively. According to genetic similarity and intraspecies differentiation, the eight Staphylococcus isolates were grouped into two main different clusters with about 58% genetic similarity. Interestingly,

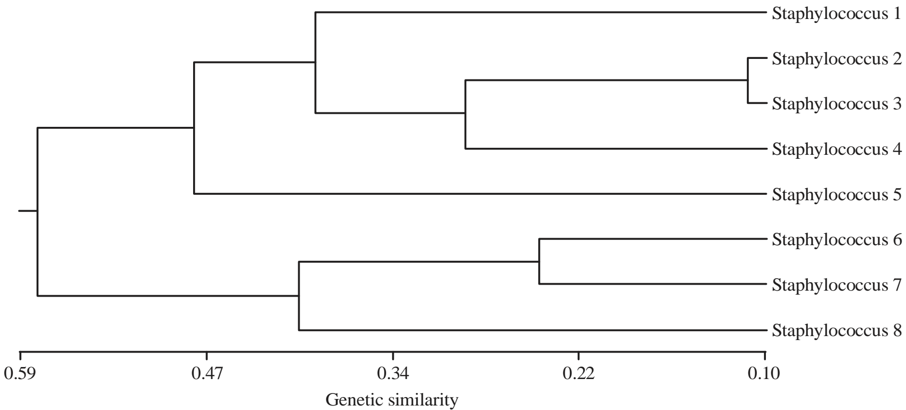

Fig. 8: Dendrogram analysis among the eight antibiotic resistance Staphylococcus isolates based on the six repetitive sequence primers

Table 3: Polymorphism level detected by the six repetitive sequence primers that have been used for Rep-PCR analysis

Primers name	Total bands	Monomorphic bands	Polymorphic bands	Monomorphism (%)	Polymorphism (%)
BOX A1	26	5	21	19.20	80.8
$(GTG)_5$	25	1	24	4.00	96.0
REP1R-I	17	4	13	23.50	76.5
MBO-REP1	13	1	12	7.60	92.4
REP2-II	15	3	12	20.00	80.0
REP8-I	12	4	8	33.30	66.7
Total	108	17	91	15.30	84.7

the first cluster was divided to two sub-clusters and Staphylococcus 6 isolate was found to be alone in the first sub-cluster, while, Staphylococcus 1, 2, 3 and 4 were grouped in the second sub-cluster. On the other hand, the other three Staphylococcus isolates were grouped in the second cluster (Fig. 8 and Table 3).

The Rep-PCR technique was proved to be useful genetic markers used for antibiotic resistance bacteria fingerprinting. Although, major bands from Rep-PCR reactions are highly reproducible, minor bands could be difficult to repeat due to repetitive sequence priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers[36,37]. The use of multiple primer sets in Rep-PCR analysis can be used as a rapid method for preliminary biotyping of multidrug resistant strains. In a previous study using different repetitive sequence primers, the discriminatory power of Rep-PCR and its ability to characterize strains was demonstrated[24,36].

CONCLUSION

In the present study, we characterised *Staphylococcus* spp. Stains that isolated from different hospitals in Taif region, KSA, using different molecular technique. The results clearly state that the antibiotic resistance genes of *mec*A and SCCmec are verified in the Staphylococcus isolates using primer specific PCR technique.

Significance statement: The PCR-dependent assay technique is offers a rapid, simple and accurate identification of antibiotic resistance gene profiles. Therefore, it could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic resistance determinants in epidemiological studies.

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