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Phylogenetic Analysis of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) in methicillin-resistant coagulase-negative *Staphylococcus epidermidis* Associated with Different Clinical Infections in Diyala Governorate

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Abstract

Staphylococcus epidermidis is classified as one of the coagulase-negative *Staphylococci* (CONS), widely acknowledged as significant nosocomial pathogens linked to infections caused by medical devices that remain inside the body. This study aims to detect phenotyping and genotyping of Methicillin resistance by cefoxitin test and *mec*A, among healthcare-associated infections and then detect genotyping of SCC, *PVL* and aminoglycoside genes. Twenty-seven isolates were obtained from 222 specimens (burn, wound, ear, nasal, throat swabs, urine, blood, fluid, and abscesses). According to the cefoxitin test, divided methicillin-resistant (MRSE) was 17/27 (62.9%), and methicillin-susceptible (MSSE) was 10 (37.03%). Resistance to antibiotics revealed that resistance to Ceftriaxone (96.2%), Amoxilline-clavulanic acid (29.6%), Aminoglycosides group (Gentamycin, Netlimycin, Streptomycin, Kanamycin, Amikacin and Tobramycin) are 14.4%, 3.7%, 3.7%, 48.1%, 3.7% and 14.8%, respectively, Azithromycin (74%), Ciprofloxacin (33.3%) and Trimethoprim-sulfamethoxazole (70.3%), Tetracyclin

(51.8%). Antibiotic susceptibility testing of the *S. epidermidis* isolates showed that 5 (18.5%), 10 (37.03 %), and 12 (44.4%) of the isolates were MDS, MDR, and XDR, respectively. In the current study, (5) MRSE Gentamycin-resistant isolates were subjected to the polymerase chain reaction technique, and the results revealed that (80%) of isolates have *mec* A. In contrast, the result of the *PVL* gene was (0%). This result indicates that all isolates were Hospital acquired. The frequent aminoglycoside genes (*aac*(6')-*le-aph*(2"), *aph*(3')-IIIa and *ant*(4')-Ia) were 20%, 20%, and 20%, respectively. In the present investigation, multiplex PCR showed that SCC*mec* type I was the predominant type. Most isolates 3/5 (60%) were type I, followed by 2/5 (40%) were non-typeable. Regrettably, MRSE isolates of the SCCmec types II or III, IV, and V could not be identified, perhaps due to the limited number of analyzed isolates. The results of typing 5 isolates of extensively drug-resistant MR-*Staph. Epidermidis* indicated the presence of 4 distinct clones, and one isolate was unique. It is considered the parent isolate for other cloned isolates.

Keywords: Gentamicin resistance, Methicillin-resistant *Staphylococcus epidermidis, PVL* gene, Staphylococcal cassette chromosome *mec*, PCR.

Introduction

Coagulase-negative *Staphylococci* (CoNS), especially *Staphylococcus epidermidis*, are the main pathogens of nosocomial bacteraemia linked to catheter and neonatal sepsis. High morbidity and mortality rates are related to these infections, primarily when CoNS develop resistance to semisynthetic β-lactam antibiotics like methicillin [1]. Additionally, these bacteria show resistance to several drugs and can pose significant challenges in public health systems. Antibiotic resistance in CoNS can be caused by several causes, including biofilm development, the presence of resistant genes, excessive use, improper use, and poor prescription of antibiotics [2]. Methicillin resistance in *S. epidermidis* is frequently linked to resistance against other antibiotics, including aminoglycosides, rifampicin, erythromycin, and trimethoprim-sulphamethoxazole [3]. Aminoglycosides are broad-spectrum bactericidal antibiotics of high potency traditionally used to treat serious and life-threatening Gram-negative and some Gram-positive infections. Aminoglycosides treat serious infections caused by Gram-positive bacteria in various parts of the world, including Iraq [4]. The prevalence of antimicrobial agent

resistance in *Staphylococci* applies primarily to plasmid or transposon acquisition. Conjugative plasmids are commonly used in Staphylococci to facilitate the conjugative transfer of resistance determinants between species and genera [1].

The main factor of Staphylococcal bacteria's resistance to methicillin and other antibiotics is the existence of the *mec* gene. A gene is a unit of heredity that is responsible for the transmission of traits from parents to offspring. This gene is derived from a sizable movable genetic element on the chromosome. The *Staphylococcus* chromosomal cassette *mec* (SCC*mec*) is a genetically movable element [5]. The SCC*mec* is a mobile genetic element specific for *Staphylococcus* associated with methicillin resistance activity[6]. Standard sequencing and molecular cloning of SCC *mec* have been used to confirm the existence and structure of a new SCC*mec* type. At the same time, PCR-based SCC*mec* identification methods have been widely employed in practical settings for an extended period. Furthermore, the utilization of whole-genome sequencing has become prevalent, leading to the recent identification of numerous SCC*mec* and comparable structures in multiple species [7]. The study aimed to determine the molecular diversity of Staphylococcal cassette chromosome *mec* (SCC*mec*) in methicillin-resistant *Staphylococcus epidermidis* and their relationship to resistance to Aminoglycoside antibiotics and another group of antibiotics in Diyala Governorate.

Materials and Methods

Isolation and identification of bacterial isolates

A total of 222 clinical specimens were collected from various sources, including (burn, wound, ear, nasal, throat swabs, urine, blood, fluid and abscesses) from patients in Baqubah City between September 2023 and January 2024. After obtaining a single colony of isolated bacteria, the isolates were identified depending on phenotypic colony characteristics, including size, shape, mannitol fermentation, and Novobiocin disc diffusion. Vitek 2 Compact System identified all isolates.

Antibiotic Susceptibility of *Staph. epidermidis*

On Muller-Hinton agar, the Kirby-Bauer method [8], was used to assess susceptibility to Twelve antibiotics, as follows: Ceftriaxone (30µg), Amoxilline-clavulanic acid (20/10µg), Gentamycin (10µg), Netlimycin (30µg), Streptomycin (10µg), Kanamycin (30µg), Amikacin

(30µg) and Tobramycin (10µg), Azithromycin (15µg), Ciprofloxacin (5µg) and Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Tetracyclin (30µg), conforming to clinical laboratory standards institute [9]. The results were recorded, and the resistance and susceptible isolates were determined by measuring the diameter of the inhibition zone according to the Clinical and Laboratory Standards Institute CLSI (2022).

Detection of biofilm

The test for biofilm formation was conducted using the microtiter plate method described by Ghellai *et al*. [10]. An ELISA reader was used to measure the concentration at 630 nm. The control well's OD value was subtracted from all the test OD values (ODc). Based on absorbance, the results were categorized into three groups: non-biofilm "OD" ≤ "OD", moderate "ODc < OD ≤ 2 x ODc", and strong "2 x ODc < OD".

Genotyping study of *S. epidermidis*

DNA template was prepared using a boiling method described by Ali *et al*. [11]. After the overnight growth of bacteria, five isolated colonies were suspended thoroughly in 2 ml distilled water and boiled in a water bath for 10 min. After centrifugation, the supernatant was used as template DNA for the PCR. All gentamicin-resistant isolates were screened by standard PCR conventional using specific primers, as shown in Table (1).

The final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, and finally, 5.5 µl nuclease-free water) in uniplex PCR Eppendorf tubes, but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction. The program used for each multiplex PCR mixture is illustrated in Table (2).

Table 1: Primers Oligonucleotide Sequences

Gene	Sequences (5' → 3')	Product Size/bp	Reference
PVL	ATCATTAGGTAAAATGTCTGGACATGATCA	433	
	GCATCAAGTGTATTGGATAGCAAAAGC		
*mec*A	GTAGAAATGACTGAACGTCCGATAA	310	
	CCAATTCCACATTGTTTCGGTCTAA		
aac(6')-Ie-aph(2")-Ia	CAGAGCCTTGGGAAGATGAAG	348	
	CCTCGTGTAATTCATGTTCTGGC		
aph(3´)-IIIa	GGCTAAAATGAGAATATCACCGG	523	

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	CTTTAAAAAATCATACAGCTCGCG		
ant(4´)-Ia	CAAACTGCTAAATCGGTAGAAGCC	294	[12]
	GGAAAGTTGACCAGACATTACGAACT		
ccrA2-B	ATTGCCTTGATAATAGCCYTCT	937	
	TAAAGGCATCAATGCACAAACACT		
ccrC	CGTCTATTACAAGATGTTAAGGATA	518	
	CCTTTATAGACTGGATTATTCAAAA		
IS1272	GCCACTCATAACATATGGAA	415	
	CATCCGAGTGAAACCCAAA		
*mec*A-*IS431*	TATACCAAACCCGACAACTAC	359	
	CGGCTACAGTGATAACATCC		

Table 2: Amplification program of primers

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
PVL	**95°C/ 5min**	**35**	**95°C/ 30 sec**	**53°C/1min**	**72°C/1min**	**72°C/7min**
*mec*A	**95°C/ 5min**	**35**	**95°C/ 30 sec**	**53°C/1min**	**72°C/1min**	**72°C/7min**
aac(6')-Ie-aph(2")-Ia	**95°C/ 7min**	**35**	**94°C/ 40 sec**	**53°C/40 sec**	**72°C/40 sec**	**72°C/2min**
aph(3´)-IIIa	**94°C/ 3min**	**35**	**94°C/ 40 sec**	**53°C/40 sec**	**72°C/40 sec**	**72°C/2min**
ant(4´)-Ia	**94°C/ 3min**	**35**	**94°C/ 40 sec**	**53°C/40 sec**	**72°C/40 sec**	**72°C/2min**
ccrA2-B	**94°C/ 5min**	**35**	**94°C/ 1min**	**55°C/1min**	**72°C/1min**	**72°C/10min**
ccrC	**94°C/ 5min**	**35**	**94°C/ 1min**	**55°C/1min**	**72°C/1min**	**72°C/10min**
IS1272	**94°C/ 5min**	**35**	**94°C/ 1min**	**55°C/1min**	**72°C/1min**	**72°C/10min**
mecA-IS431	**94°C/ 5min**	**35**	**94°C/ 1min**	**55°C/1min**	**72°C/1min**	**72°C/10min**

Statistical analysis

A statistical analysis was conducted using either Pearson's chi-square test or Fisher's exact test, depending on the nature of the contingency table and the qualitative variables involved. A correlation analysis using either Pearson's or Spearman's correlation coefficient was conducted to assess the relationship between the quantitative variables, taking into account their distribution [13]. The correlation and display of the results were conducted using the ggplot2 software [14].

Results and Discussion

Distribution of *S. epidermidis* according to the source of Infection

Twenty-seven primary clinical isolates of gram-positive bacteria were identified as *Staph. epidermidis* using macroscopic, microscopic, and biochemical assays, which were confirmed

genetically by Vitek 2 Compact. The source of these specimens was as follows: 8 isolates from blood, 7 isolates from burns, 5 isolates from urine, 4 isolates from wounds, 2 from ear swabs, and 1 isolate from nasal swabs, while abscesses, throat swabs, and fluid samples were negative growth. The highest percentage of 8/43 (18.6%) of positive isolates was obtained from blood samples, while the lowest isolation rate was 3.7% from nasal swabs.

Antimicrobial Sensitivity Test

In this study, out of 27 clinical isolates, (17) isolates appeared as MRSE, and (10) isolates appeared as MSSE according to complete resistance toward Cefoxitin. According to phenotype, 96.2% and 29.6% of *Staph. epidermidis* showed resistance to Ceftriaxone and Amoxicillin-clavulanic acid, respectively. This study, in agreement with Behshood *et al*. [15], was resistant to Ceftriaxone (85%). While these results disagree with the analysis performed by Shrestha *et al.* [16], which discovered resistance to Ceftriaxone was (58%). Beta-lactams are bactericidal agents that kill bacteria by obstructing the formation of the cell wall in both Gram-negative and Gram-positive bacteria. They achieve this by interacting with PBPs, which hinders the transpeptidation reaction, resulting in the rupture and demise of the bacterial cell. Bacterial resistance is becoming an increasing problem in both community and healthcare settings due to the extensive usage of these antibiotics [17].

The resistance of *S. epidermidis* isolates to the aminoglycoside group, which included Gentamycin 14.8%, Netlimycin 3.7%, Streptomycin 3.7%, Kanamycin 48.1%, Amikacin 3.7%, Tobramycin 14.8%, as shown in Table (3). These results disagree with Farajzadeh Sheikh *et al*. [18], which reported higher resistance rates, with 52% of *S. epidermidis* isolates being resistant to Amikacin and 46%, 33%, of *S. epidermidis* isolates showing resistance to Gentamicin and Tobramycin, respectively. Also, these results disagree with the study performed in Egypt by Moawad *et al.* [19], who found the resistance rates for Gentamicin and Amikacin were 79.5% and 74.4%, respectively. Decades of data from *in vitro* biochemical and structural research indicate that aminoglycoside drugs attach to the bacterial ribosome and disrupt protein synthesis by inhibiting the ribosome's translocation on the messenger RNA and inducing miscoding mistakes. Nevertheless, there is limited understanding regarding the dynamic effects of these compounds on intracellular protein synthesis [20].

Table 3: Antibiotic Susceptibility profile of *Staphylococcus epidermidis* isolates (n=27).

Antibiotic	Resistance	Sensitive
Ceftriaxone	26(96.2%)	1(51.8%)
Amoxicillin-clavulanic acid	8(29.6%)	19(70.3%)
Gentamycin	4(14.8%)	23(70.3%)
Netlimycin	1(3.7%)	26(70.3%)
Streptomycin	1(3.7%)	26(96.2%)
Kanamycin	13(48.1%)	14(51.8%)
Amikacin	1(3.7%)	26(96.2%)
Tobramycin	4(14.8%)	23(85.1%)
Azithromycin	20(74%)	7(25.9%)
Ciprofloxacin	9(33.3%)	18(66.6%)
Trimethopri-sulfamethoxazole	19(70.3%)	8(29.6%)
Tetracyclin	14(51.8%)	13(48.1%)

The current results indicate that 74% and 70.3% of *S. epidermidis* isolates resist Azithromycin and Trimethoprim-sulfamethoxazole, respectively. The current results differ from a study conducted in southwest Iran, which reported lower resistance rates for *S. epidermidis* against Azithromycin and Trimethoprim-sulfamethoxazole (13%) [18]. Also, these results disagree with the study performed by Thomas *et al.* [21], which discovered resistance to Azithromycin and Trimethoprim-sulfamethoxazole was 61.2% and 28%, respectively. Azithromycin, a kind of macrolide, specifically binds to the 50S ribosome and inhibits the process of protein synthesis. Resistance arises due to different causes, including mutations in the 23S rRNA and protein L4, methylation of the 23S rRNA, and the presence of efflux systems Mef (A) and Msr (A) [22]. The current study differs from a study conducted by Pandey *et al*. [23], which reported resistance rates of 80% for Ciprofloxacin and 60% for Tetracycline. These findings are consistent with an Italian study by Petrillo *et al*.[24], which found a resistance rate of 58.5% for Tetracycline.

Combination antibiotics of *Staphylococcus epidermidis*

The results of the current study showed a positive correlation between aminoglycoside antibiotics and the *β*-Lactams group (Amoxicillin-clavulanic acid, and Ceftriaxone) and a strong positive correlation between aminoglycoside antibiotics and Tetracyclin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, and Azithromycin. Also, a positive correlation exists between

Tetracyclin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, and Azithromycin, Figure (1). Combination antimicrobial therapy is a recognized approach for treating serious bacterial infections in both human and veterinary medicine. Concomitantly providing two or more antimicrobials, which normally work through separate molecular mechanisms, expands the range of activity and has the potential to achieve synergistic bacterial eradication, as opposed to using a single drug. Moreover, the utilization of antimicrobial combinations can be advantageous in promoting the breakup of biofilms and inhibiting the establishment of antimicrobial resistance. This, in turn, aids in the preservation of last-resort drugs [25].

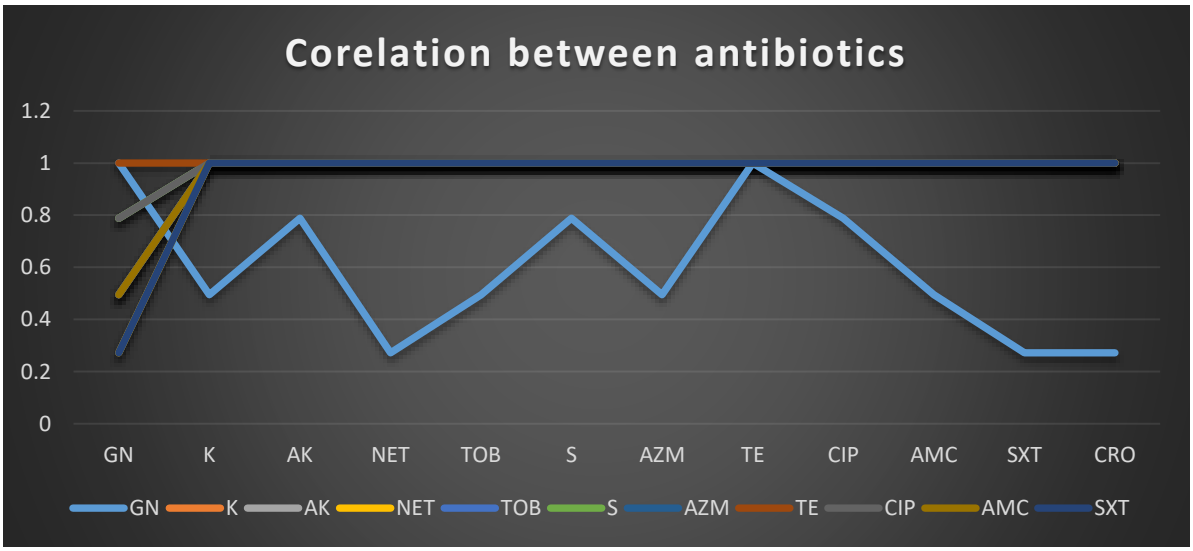

Figure 1: Correlation between antibiotics.

Detection of Biofilm by Microtiter plate method

The current findings reveal that 18 out of 27 (66.6%) *S.epidermidis* isolates were classified as moderate biofilm formation, while 9 out of 27 (33.3%) were categorised as strong biofilm formation when assessed using the microtiter plate method. The result of the current study disagreed with Farajzadeh Sheikh *et al*. [18], who found that (45%) were categorized as strong biofilm-formers and (32%) were moderate, while (21%) were weak biofilm-formers, while another study reported in Northern Iran by Kord *et al*. [26], who found 53.6% of *S. epidermidis* isolates were able to produce biofilm. The capacity to form biofilms differed for each isolate because several factors influence the capacity to form biofilms, such as the medium, the

detection method, the incubation condition, oxygen concentration, nutrient level, and microbial community [27].

Relationship between the antibiotic susceptibility status and biofilm production

The correlation between Biofilm production scores and antibiotic susceptibility appears non-statistically significant (*P*-value is more significant than 0.05) for all antibiotics used in this study, as shown in Table (4). The result showed a negative correlation, as most of the resistance to antibiotics was in moderate biofilm formation.

Tables 4: Antibiotic resistance pattern correlated with biofilm production

Antibiotics	Susceptibility status %	Moderate n=18	Strong n=9	Total formation n=27	P- value
Gentamicin	R	3(16.6)	1(11.1)	14.8	0.31
	S	15(83.3)	8(88.8)	85.1%	0.14
Kanamycin	R	8(44.4)	5(55.5)	48.1%	0.40
	S	10(55.5)	4(44.4)	51.8%	0.10
Amikacin	R	1(5.5)	0(0)	3.7%	0.31
	S	17(94.4)	9(100)	96.2%	0.11
Netlimicin	R	1(5.5)	0(0)	3.7%	0.31
	S	17(94.4)	9(100)	96.2%	0.11
Tobramycin	R	2(11.1)	2(22.2)	14.8%	1
	S	16(88.8)	7(77.7)	85.1%	0.06
Streptomycin	R	1(5.5)	0(0)	3.7%	0.31
	S	17(94.4)	9(100)	96.2%	0.11
Azithromycin	R	12(66.6)	8(88.8)	74.07%	0.37
	S	6(33.3)	1(11.1)	25.9%	0.06
Tetracyclin	R	12(66.6)	3(33.3)	55.5	0.067
	S	6(33.3)	6(66.6)	44.4%	1
Ciprofloxacin	R	5(27.7)	4(44.4)	33.3%	0.73
	S	13(72.2)	5(55.5)	66.6%	0.06
Amoxicillin-clavulanic acid	R	4(22.2)	4(44.4)	29.6%	1
	S	14(77.7)	5(55.5)	70.3%	0.1082
Trimethopri-sulfamethoxazole	R	13(72.2)	6(66.6)	70.3%	0.10
	S	5(27.7)	3(33.3)	29.6%	0.47
Ceftraxion	R	17(94.4)	9(100)	96.2%	0.11
	S	1(5.5)	0(0)	3.7%	0.31

Molecular detection by Polymerase Chain Reaction(PCR) in *S. epidermidis*

Among 17 MRSE isolates tested, 5 were identified as extensively drug-resistant (XDR) and resistant to Gentamycin and were utilized for the genotyping study.

Detection of *mec* A gene

Among 5 MRSE, 80% (4/5) possess the *mec* A gene, Figures (2). These results disagree with the study performed by [18], who found (53%) of *S. epidermidis* isolates had the *mec*A gene. While, this result agrees with the study performed by Rocchetti *et al* . [28], who found (78.7%) were positive for the *mec*A gene. The presence of the *mec*A gene in methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* is the main reason for their resistance towards antibiotics derived from penicillin, such as Methicillin and Cefoxitin. This gene encodes for penicillin-binding protein (PBP2a), which reduces the binding affinity of the bacteria towards β-lactam antibiotics [29].

Relationship between the presence of *PVL* gene and SCC*mec* typing

One of the virulence genes expressed by methicillin-resistant Staphylococci is panto-valentine leukocidin (*PVL*), which is known to be related to a severe type of community-acquired MRSA infection. In contrast, the prevalence rate of *PVL* is usually low in Methicillin susceptible *Staphylococci* and healthcare infection [30]. The *PVL* gene was detected in (0 %) of the MRSE Figure (2). This indicates that all isolates were acquired by the hospital. At the same time, only one SCC*mec* class was identified. Most of the isolates 3/5 (60%) were type I, followed by 2/5 (40%) were non-typeable, Figure (3). We couldn't identify MRSE isolates of the SCC*mec* types II or III, IV and V. To our knowledge, we are the first study describing the SCCmec typing in our governorate with such a high prevalence of SCCmec type I. The results of the current study are not in concordance with a study conducted in India by Nagasundaram and Sistla [31], who found the predominant SCCmec type was type III (62%), followed by type V (52.5%) and type I (47.5%), while a single isolate carried type II. Also, Some isolates (40% of the total) were not typeable using this method, similar to what previous studies have reported from Iran and other regions of the globe [32]. These isolates may convey other SCCmec types (e.g., VI or XI SCCmec types) and/or unknown types not investigated in our study.

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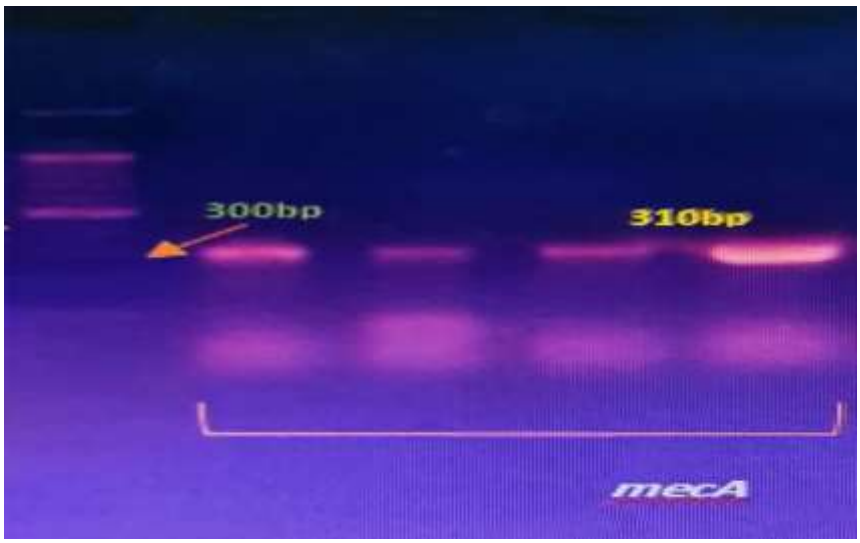

Figure 2: Agarose gel electrophoresis (1% agarose,7v/cm2 for 60 min) for *pvl* gene (433bp amplicon) and *mec*A gene (310bp amplicon) lane M100bp DNA Ladder, lanes 1-12 represent of bands.

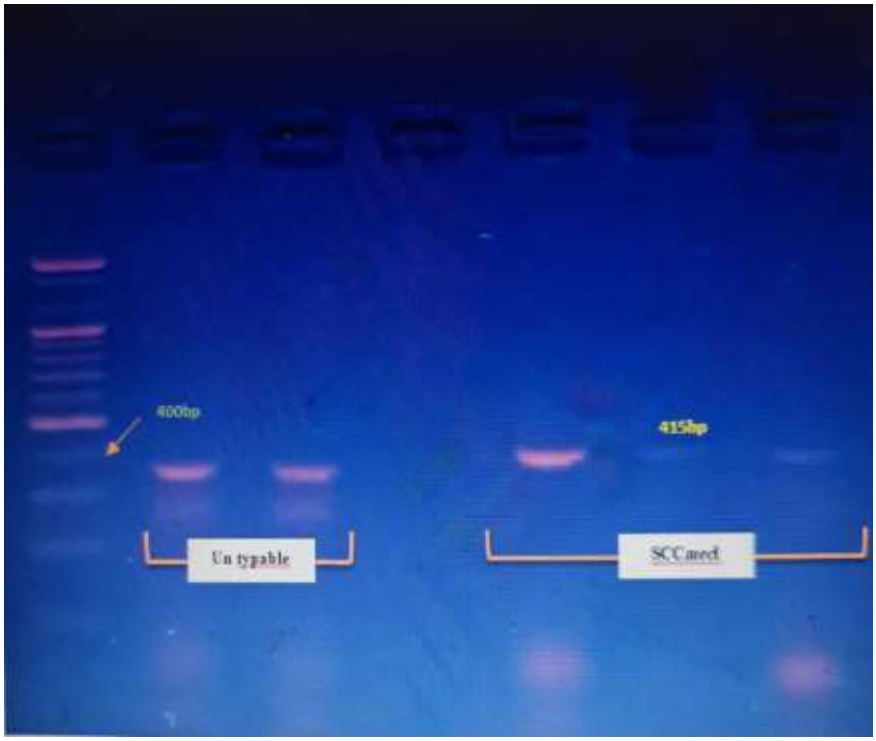

Figure 3: Agarose gel electrophoresis (1% agarose,7v/cm^2 for 60 min) for SCC*mec* types, lane M100bp DNA Ladder, lanes 4-6 represent of SCC*mec* I (IS1272 bands only) and lanes 1-2 represent of untypable SCC*mec*.

Detection of aminoglycoside genes

The results of Multiplex-PCR indicated that the frequency of *aac*(6')-*Ie*-*aph*(2"), *aph*(3')-IIIa, and *ant*(4')–Ia genes among 5 isolates were 20%, 20%, and 20%, respectively. The evaluation of the prevalence of antibiotic resistance and the use of molecular typing techniques for the identification of genes responsible for resistance can help find ways to control these bacteria efficiently and reduce hospital-acquired infections caused by *Staphylococci* [33].

Relationship between phenotypic and genotypic characteristics of Methicillin- resistance *Staphylococcus epidermidis*

The results of the current study suggest potential associations between aminoglycoside antibiotic resistance and the presence of the *mec* A gene in *S. epidermidis* isolates. Among 5 MRSE isolates, all isolates were resistant to Kanamycin (100%), while 60% were resistant to Gentamicin and possessed the *mec* A gene. This indicates a positive correlation between Gentamicin resistance and the *mec* A gene. On the other hand, there is a strong positive correlation between Gentamicin resistance and aminoglycosides genes (*aac*(6')-*Ie*-*aph*(2"), *aph*(3´)-IIIa). At the same time, there is a negative correlation between other aminoglycosides antibiotics and aminoglycosides genes and a negative correlation between aminoglycosides genes and other antibiotics used in this study. Also, there is a negative correlation between aminoglycosides antibiotics and SCC*mec* class I (IS1272), Figure (4).

Phylogenetic and Molecular Profile of Methicillin-resistant *Staphylococcus epidermidis* Isolated from clinical samples

The primary objective was to assess the genetic variability among 5 isolates of extensively drug-resistant MR-*Staph. epidermidis* obtained from burns, wounds, ear, nasal, throat swabs, urine, blood, fluid, and abscesses. The results indicated the presence of 4 distinct clones, which were further categorized into two main clusters: cluster I and cluster II. Cluster I consisted of two isolates (SE4, SE25), and isolates 4 and 25 were 0.6% identical to each other. Furthermore, cluster II included two isolates (SE9, SE23). These isolates were 0.5% identical to each other. At the same time, one isolate was found to be unique (SE16), with similarity coefficients below 0.5 (Unique clone), Figure (5).

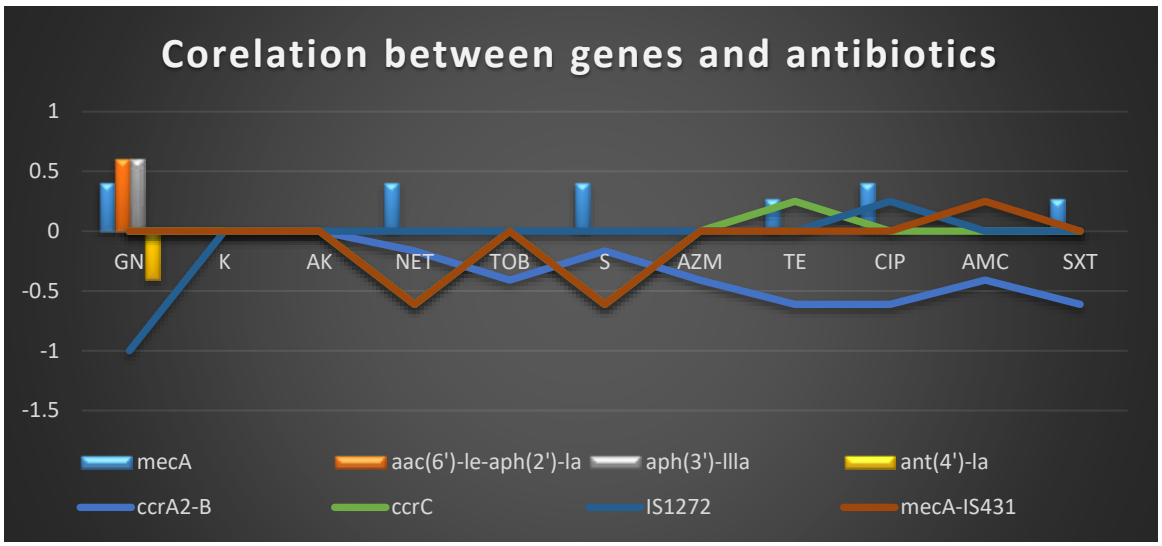

Figure 4: Correlation between genes and antibiotics of *Staphylococcus epidermidis*

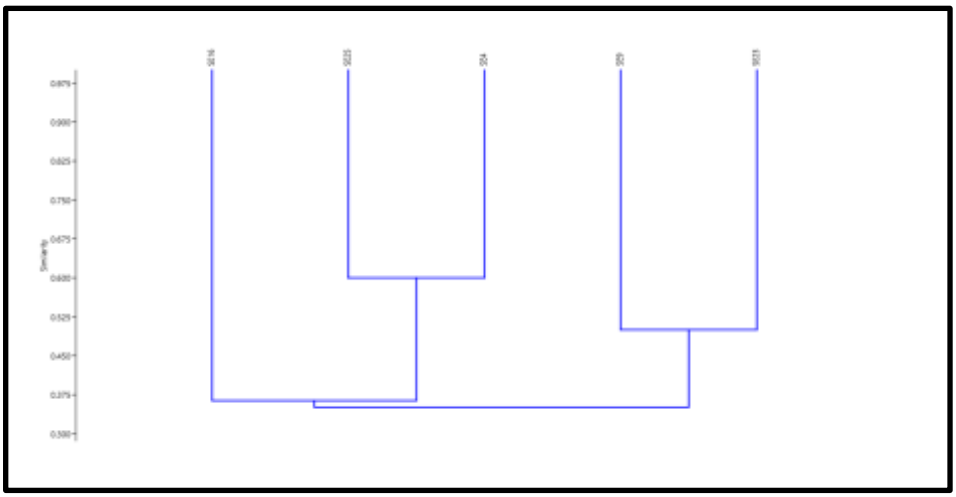

Figure 5: Dendrogram analysis for 5 clinical *Staphylococcus epidermidis* isolates, by Jaccard coefficient values with Arithmetic Mean UPGMA.

Relationship between Antibiotic Susceptibility Test and Phylogeny among clinical Isolates

The relationship between phenotypic characters (susceptibility to antibiotics and biofilm formation capacity) and Phylogeny among 27 isolates of *S. epidermidis* indicate the high variability in the patterns obtained in Figure (6).

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The results showed the presence of 26 distinct clones, which were further categorized into two main clusters: Cluster I consisted of four isolates (9, 15, 10, 23), while cluster II can be divided into two groups: Group A consisted of four isolates (4, 8, 25, 26), and group B included: Sub-group I consisted of seven isolates (2, 24, 27, 19, 5, 20, 21) and Sub-group II consisted of ten isolates (18, 3, 14, 17, 6, 13, 11, 22, 7, 12) and Unique clone (isolate 1). At the same time, Among 27 isolates, one unique isolate (isolate 16) was considered the original from which the isolates in both clusters I and II were cloned.

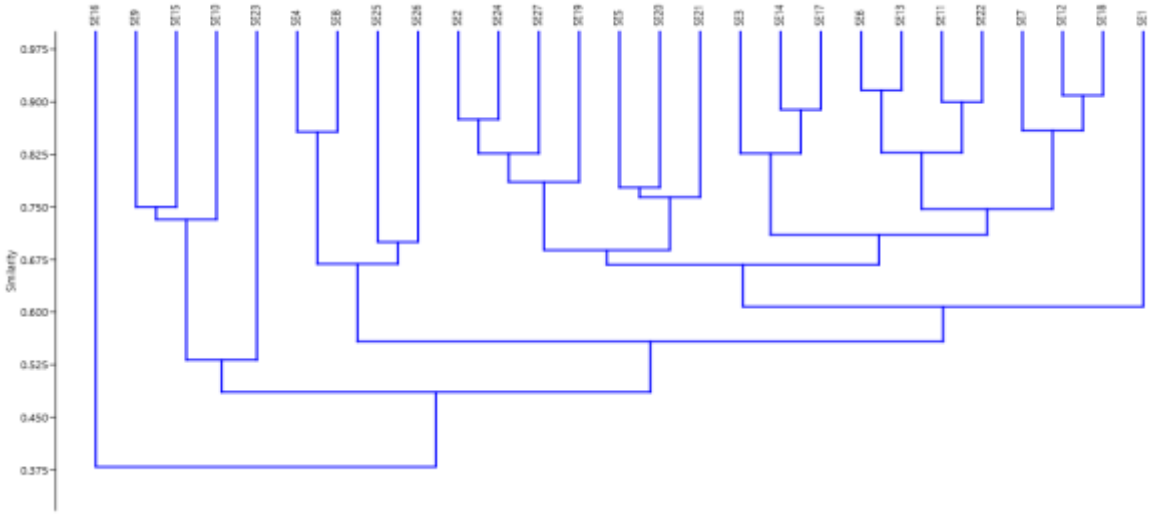

Figure 6: Dendrogram analysis for clinical *Staphylococcus epidermidis* isolates, by Jaccard coefficient values with Arithmetic Mean UPGMA.

Isolates within cluster I showed resistance 100% to Kanamicin, Azithromycin, Ciprofloxacin, Amoxicillin-clavunic acid, and Ceftriaxone. While isolates within cluster II, group A showed resistance 100% to Kanamicin, Tetracyclin, and Ceftriaxone and isolates within group B (sub-group I) showed resistance 100% to Tetracyclin, Trimethoprim-sulfamethoxazole, Ceftriaxone. Isolates within group B (sub-group II) No complete resistance to any antibiotic used in this study was observed. In contrast, Isolate 16 (parent isolate) showed resistance 100% to Gentamicin, Kanamicin, Tobramycin, Streptomycin, Azithromycin, Tetracyclin, Trimethoprim-sulfamethoxazole, Ceftriaxone, as shown in Table (5). At the same time, all isolates could form biofilm. These results indicate that isolate 16 exhibited the highest antibiotic

resistance compared to the isolates within group B (sub-group II), which showed lower resistance. This suggests that the further the isolate gets from the parent isolate in the phylogeny tree, the greater the genetic diversity between the isolates.

Table 5: The Relationship between Antibiotic Susceptibility Test and Phylogeny among Isolates.

Antibiotics	Susceptibility state	Phylogenetic groups					
		Cluster I	Cluster II				Unique isolate
			Group A	Group B			
				Sub-group I	Sub-group II	Unique isolate	
Gentamycin	Resistance	25%	25%	14.2%	0%	0%	100%
	sensitive	75%	75%	85.7%	100%	100%	0%
Kanamycin	Resistance	100%	100%	57.1%	0%	0%	100%
	sensitive	0%	0%	42.8%	100%	100%	0%
Amikacin	Resistance	0%	0%	0%	0%	100%	0%
	sensitive	100%	100%	100%	100%	0%	100%
Netlimicin	Resistance	0%	0%	14.2%	0%	0%	0%
	sensitive	100%	100%	85.7%	100%	100%	100%
Tobramicin	Resistance	50%	0%	14.2%	0%	0%	100%
	sensitive	50%	100%	85&%	100%	100%	0%
Streptomycin	Resistance	0%	0%	0%	0%	0%	100%
	sensitive	100%	100%	100%	100%	100%	0%
Azithromycin	Resistance	100%	0%	71.4%	80%	100%	100%
	sensitive	0%	100%	28.5%	20%	0%	0%
Tetracycline	Resistance	25%	100%	100%	30%	0%	100%
	sensitive	75%	0%	0%	70%	100%	0%
Ciprofloxacin	Resistance	100%	75%	0%	10%	100%	0%
	sensitive	0%	25%	100%	90%	0%	100%
Trimethoprim-sulfamethoxazole	Resistance	75%	50%	100%	40%	100%	100%
	sensitive	25%	50%	0%	60%	0%	0%
Amoxicillin-clavulanic acid	Resistance	100%	25%	0%	30%	0%	0%
	sensitive	0%	75%	100%	70%	100%	100%
Ceftriaxone	Resistance	100%	100%	100%	90%	100%	100%
	sensitive	0%	0%	0%	10%	0%	0%

Conclusions

The prevalence of hospital-acquired MRSE was high. SCC*mec* type I was predominant, and the *pvl* gene did not play any role in the virulence of the MRSE strains isolated from hospitals in Baqubah City. In addition, current data revealed a high frequency of gentamicin resistance among the HA-MRSE isolates, with the predominance of *aac*(6')-*le*-*aph*(2"), *aph*(3')-IIIa, and *ant*(4′)-Ia aminoglycoside-resistant determinants.

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