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Original Article

Mobile Genetic Elements in Coagulase Negative Staphylococcus Isolated from Nosocomial Infections

Mohammed Obaid Ibrahim[*1,2], Harith Kamil Buniya[3], Ali Hazim Abdulkareem[4]

[1]*Department of biology, college of science, University of Anbar, Ramadi 31001, Iraq.*
[2]*Department of Medical Laboratory Techniques, Al-Huda University College, Ramadi 31001, Iraq.*
[3]*Department of Biology, College of Education for Pure Science, University of Anbar, Ramadi 31001, Iraq.*
[4]*Department of biotechnology, college of science, University of Anbar, Ramadi 31001, Iraq.*

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ABSTRACT

The presence of SCCmec components in coagulase-negative staphylococci (MR-CoNS) increases their resistance to methicillin and other antibiotics, making them difficult to treat. This study aims to investigate the distribution of species, antimicrobial susceptibility patterns, and genetic characteristics of methicillin-resistant coagulase-negative staphylococci (MR-CoNS) isolates from clinical settings, with a particular emphasis on the presence and distribution of SCCmec types. A total of 50 MR-CoNS isolates were collected and identified, and their species distribution was determined. The Kirby-Bauer disc diffusion test was used to assess antimicrobial susceptibility. SCCmec typing was performed to categorize the isolates, and sequencing analysis was conducted to compare their genetic elements with worldwide isolates in GenBank. Staphylococcus haemolyticus (60%) was the most common isolate, followed by Staphylococcus epidermidis (40%). All isolates exhibited complete resistance to cefotaxime, augmentin, amoxicillin, oxacillin, and ceftazidime. SCCmec type I demonstrated the highest resistance, while types II to V showed varied patterns. Sequencing analysis revealed strong genetic similarities between clinical and global isolates, with similarity rates ranging from 99.89% to 100%. After sequencing analysis, 12 genes were deposited in GenBank under the accession numbers specific to this study: PQ118407, PQ151368, PQ151369, PQ151370, PQ151371, OR619697, PP990495, PP990496, PQ037176, OR619696, PQ151372, and PQ205317. This study underscores significant antibiotic resistance in MR-CoNS, particularly in S. haemolyticus and S. epidermidis. The high resistance observed in SCCmec type I strains highlights the need for vigilant monitoring and targeted therapies. Furthermore, the genetic similarities between clinical and global isolates emphasize the importance of global surveillance and the development of new antimicrobial strategies to combat MR-CoNS infections.

Keywords: SCCmec types, MR-CoNS, antimicrobial susceptibility pattern, mobile genetic elements.

1 Introduction

Coagulase-negative staphylococci (CoNS), typically part of the normal flora on mammalian skin and mucous membranes, are known to cause nosocomial infections[1]. They can form biofilms, enabling them to adhere to medical devices and cause infections such as foreign body-related infections, infections in "preterm newborns, and endocarditis"[2]. These biofilm-producing CoNS are increasingly reported to be resistant to methicillin and various antibiotic classes, including lincosamides and macrolides. This growing resistance poses a significant challenge in treating clinical infections, complicating antibiotic selection for patient care[3]. The most commonly isolated MR-CoNS species in hospitals are *S.epidermidis* and *S.haemolyticus*[4]. These organisms have the mecA gene, which produces a protein called PBP2a. PBP2a makes these organisms less sensitive to beta-lactam antibiotics[1]. The *mecA* gene, acquired through the Staphylococcal Cassette Chromosome *mec* (SCCmec), a mobile genetic element[5]., is responsible for methicillin resistance in bacteria. It's composed of two main parts: the mec gene complex and the *ccr* gene complex. The *mec* genes confer methicillin resistance, while the *ccr* genes help in the movement of SCCmec into and out of the bacterial chromosome. This movement creates different types of SCCmec (I-XI), and a single bacterium can have multiple types[6].

In addition to these components, SCCmec also contains J regions, which are used to determine SCCmec subtypes, as well as various

* Corresponding author
E-mail address moh21s1012@uoanbar.edu.iq(Instructor).
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non-essential elements that may carry additional antimicrobial resistance genes[7]. SCCmec types III, IV, and V are commonly found in MR-CoNS[6]. Due to the limited data on MR-CoNS in Iraq, this study aims to: (1) identify the distribution of MR-CoNS species from various sources, (2) analyze their antimicrobial susceptibility profiles, (3) detect their SCCmec types, and (4) assess the antimicrobial susceptibility patterns associated with these SCCmec types. The findings, especially regarding SCCmec genes, will provide preliminary data that could support future research and aid in the management of MR-CoNS infections.

2 Methodology

Staphylococci were identified from different body samples. They were grown on a special type of agar and tested for their characteristics. A machine called VITEK 2 was used to identify the specific type of *staphylococcus.*

2.1 Detection of antibiotic resistance profile

The "Kirby-Bauer disc diffusion method" was used 19 antibiotic discs ("Oxoid, UK"), including ceftazidime, cefotaxime, gentamicin, and others. Zones of inhibition were measured and compared to CLSI standards (CLSI, 2022).

2.2 Molecular screening of mecA gene

DNA was extracted from the bacteria, and its purity was measured. A specific *mecA* gene was then tested for forward "(5′-TCCAGATTACAACTTCACCAGG-3′)" and reverse "(5′-CCACTTCATATCTTGTAACG-3′)" primers, following the method described by[35]. *Staphylococcus epidermidis* ATCC 35984 and ATCC 12228 were used as positive and negative controls, respectively. The PCR mixture (20 µL total volume) included 10 µL master mix (Thermo Scientific, USA), 0.5 µL of each 10 µM primer, 1 µL DNA template, and 8 µL nuclease-free water. The PCR was carried out on a Bio-Rad MyCycler™ (USA) with the following settings: initial denaturation at 98°C for 30 seconds, 28 cycles of denaturation (98°C for 10 seconds), annealing (52°C for 30 seconds), and extension (72°C for 30 seconds), followed by a final extension at 72°C for (5 minutes) and a hold at 4°C. PCR products were analyzed by gel electrophoresis at 58 V for 120 minutes on a 1.4% agarose gel containing 0.5 µL gel stain (Biotech, China). A 100 bp DNA ladder (Vivantis, Malaysia) was served as a marker. The gel was visualized under UV light and imaged with a gel imager (Major Science, USA).

2.3 Typing of the Staphylococcal Cassette Chromosome *mec* (SCCmec)

A multiplex PCR test was developed to identify different types of SCCmec, which are responsible for methicillin resistance in staphylococci. The primers that target specific genes in each SCCmec type, which described in Table 1. multiplex PCR program dived in two groups depending on annealing temperature, first group consist (type I, II, and III) annealing temp.55°C, and second group (type IV and V) was 62°C.The initial denaturation step for each PCR assay with different primers was established at 95°C for 4 min. Also, the denaturation was 94°C for 30 sec. The annealing time is 50 sec. The extension time was 1 min. in 72°C. The final extension for all genes were done at 72 °C for 6min. On the other hand, the PCR reaction consisted of 12.5 µl of PCR Master Mix, 0.5 µl of each primer (forward and reverse), 7.5 µl of nuclease-free water for the first group, and 8.5 µl of nuclease-free water for the second group, along with 2 µl of the DNA template, resulting in a final volume of 25 µl. The results were analyzed using 1.5% gel electrophoresis. A DNA ladder was used to compare the sizes of the PCR products. The gel was visualized under UV light and photographed.

2.4 Sequencing of Genes

A representative strain of each successfully detected SCCmec type was sent for DNA sequencing using the ABI 3730XL automated DNA sequencer through Macrogen Company in Korea. Sequencing analysis was performed using the Basic Local Alignment Search Tool (BLASTn) available on the National Center for Biotechnology Information (NCBI) website.

2.5 Statistical Analysis

Statistical analyses were performed using a chi-square test to determine whether the distribution of resistance percentages varied significantly among SCCmec types for each antibiotic.

3 Results

3.1 Distribution of MR-CoNS

Among the 50 MR-CoNS isolates, *Staphylococcus haemolyticus* was the most frequently identified species, accounting for 60% (n = 30) of the isolates, followed by *Staphylococcus epidermidis*, which constituted 40% (n = 20), as illustrated in Figure 1.

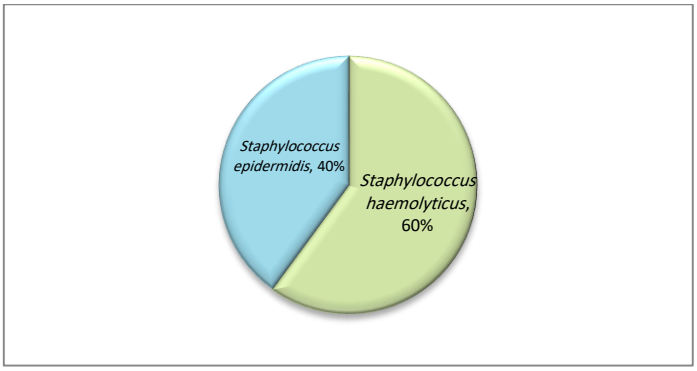

Figure 1: Distribution of methicillin-resistant coagulase-negative *Staphylococcus*, showing *Staphylococcus haemolyticus* 60% and *Staphylococcus epidermidis* 40%.

3.2 Antimicrobial susceptibility pattern

The results of the antibiotic susceptibility testing among **MR-CoNS** isolates were determined using the Kirby-Bauer disc diffusion method with antibiotic discs from Oxoid, UK. The highest resistance rate of CoNS toward amoxiclav, cefoxitin, Methicillin, Amoxillin, and ceftriaxone (100%). The isolates also demonstrated high resistance to piperacillin and ceftazidime (96%), fusidic acid (76%), tobramycin and tetracycline (68%), gentamicin (66%), and azithromycin (66%). The resistance rate of CoNS to vancomycin was 52%. Resistance levels were lower against clindamycin (46%), lincomycin and ciprofloxacin (42%), levofloxacin (40%), trimethoprim-sulfamethoxazole and rifampicin (38%), and nitrofurantoin (18%). In comparison, the lowest resistance among CoNS isolates was observed against meropenem (10%) and chloramphenicol (2%).

3.3 Molecular Screening of the *mecA* Gene

Figure 2 shows that all isolates (100%) tested positive for the detection of the *mecA* gene in methicillin-resistant coagulase-negative *Staphylococcus* isolates using uniplex PCR.

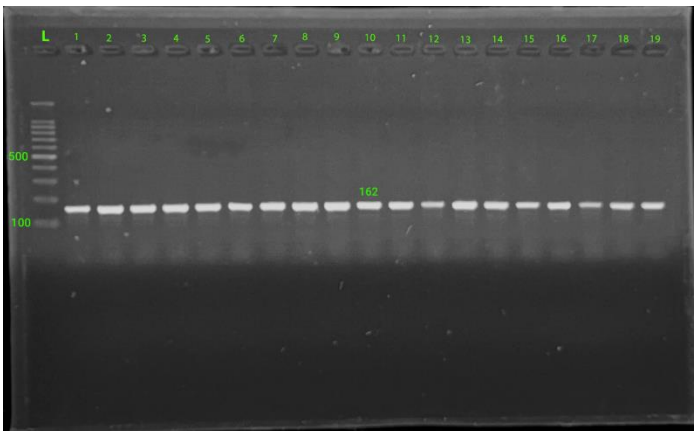

Figure 2: Uniplex PCR products for the molecular detection of the *mecA* gene in methicillin-resistant coagulase-negative *Staphylococcus* isolates, analyzed using 1.5% agarose gel electrophoresis.

3.4 Antimicrobial Sensitivity Patterns Across Staphylococcal Cassette Chromosome mec (*SCCmec*) Type

Table 2 represents antibiotic resistance patterns of Methicillin-Resistant Coagulase-Negative Staphylococcus (MRCoNS) strains, categorized by SCCmec types (I, II, III, IV, V, and non-typeable). The distribution of SCCmec types among the isolates is as follows: Type I includes 2 isolates, Type II includes 19 isolates, Type III includes 8 isolates, Type IV includes 18 isolates, Type V includes 28 isolates, and 6 isolates are non-typeable (N), as shown in Figures 3 and 4.

All isolates, regardless of SCCmec type, exhibited 100% resistance to cefotaxime, augmentin, amoxicillin, oxacillin, ceftazidime, and piperacillin, indicating that these antibiotics are ineffective against the MR-CoNS strains tested. Bacterial isolates demonstrated resistance to erythromycin, with slight variability in SCCmec III (87.5%) and SCCmec IV (94.5%). Fusidic acid resistance remained high, reaching 100% in SCCmec I and IV, while being slightly lower in SCCmec II (79%) and SCCmec V (89.2%). Tetracycline resistance ranged from 66.6% in SCCmec IV to 100% in SCCmec I and non-typeable isolates. Levofloxacin resistance varied significantly, from 37% in SCCmec II to 100% in SCCmec I. Tobramycin and ciprofloxacin exhibited similar trends, with resistance ranging from 37% to 72% in most types but reaching 100% in SCCmec I. Gentamicin resistance was lowest in SCCmec III (50%) and highest in SCCmec I and non-typeable isolates (100%). Vancomycin resistance fluctuated between 77.7% in SCCmec IV and 100% in SCCmec I. Clindamycin and lincomycin resistance followed comparable patterns, with the lowest rates in SCCmec IV (38.8% and 44.4%) and complete resistance in SCCmec I. Nitrofurantoin and imipenem showed relatively low resistance, with 0% resistance in SCCmec III and non-typeable isolates, increasing to 50% in SCCmec I. Chloramphenicol exhibited minimal resistance, detected only in SCCmec I (50%), while all other types remained fully susceptible. This study analyzes antibiotic resistance patterns across SCCmec types, revealing significant variability among antibiotic classes. Beta-lactams exhibited highest resistance (100%), indicating ineffectiveness in treatment. Resistance to macrolides, lincosamides, fluoroquinolones, aminoglycosides, glycopeptides, and tetracyclines varied significantly ($p < 0.05–0.001$), with SCCmec II, III, and IV demonstrating lower resistance in select cases. Notably, nitrofurantoin and imipenem showed highly significant differences ($p < 0.001$), with SCCmec III and V exhibiting the lowest resistance, while phenicol resistance was nearly absent except in SCCmec I. These findings highlight SCCmec type as a crucial determinant of resistance patterns, emphasizing the potential for targeted therapeutic strategies as shown Table 3.

3.5 Sequencing of *Staphylococcal Cassette Chromosome mec* (SCCmec) Typing

Table 4 provides a comparative analysis of mobile genetic elements in *Staphylococcus* isolates from this study, highlighting their genetic similarity to global isolates. The high similarity percentages suggest a close relationship between the isolates studied and those found worldwide. This finding is crucial for understanding the spread and conservation of resistance genes across different regions.

For SCCmec Type I, *Staphylococcus haemolyticus* strain 12 *ccrB1*-like gene shows 100% similarity to a global isolate in GenBank (Accession Number: CP035291.1). For SCCmec Type II, the *ccrB2*-like gene (PQ151368) shows 100% similarity to a global isolate (LR735432.1). For SCCmec Type IV, *Staphylococcus epidermidis* strain 4 *ccrB2* gene shows 99.46% similarity to a global isolate (CP120011.1). Additionally, two other *Staphylococcus epidermidis* strains, ST1183 and ST35, show 100% similarity to their corresponding global isolates (CP123738.1 and CP047851.1, respectively). For SCCmec Type V, *Staphylococcus epidermidis* strain ST23 *ccrC*-like gene shows 99.76% similarity to a global isolate (CP043845.1). For the *mecA* gene, *Staphylococcus epidermidis* strain 59 *mecA* gene shows 99.12% similarity to a global isolate (CP133663.1), as illustrated in Table 4.

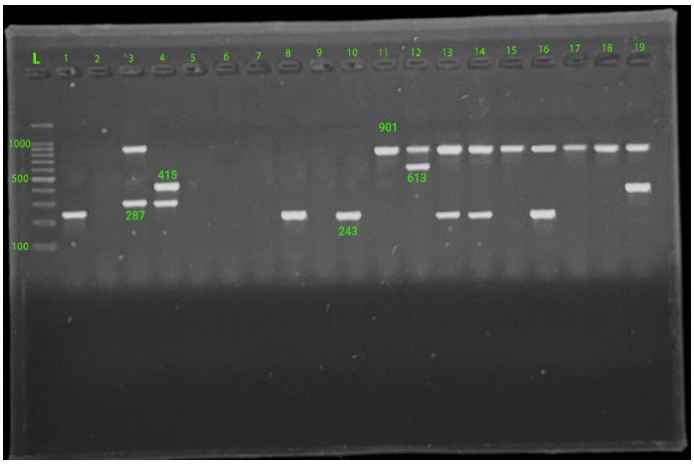

Figure 3: Multiplex PCR products of methicillin-resistant coagulase-negative Staphylococcus isolates. The distribution of SCCmec types among the isolates is as follows: Type I includes 1 isolate, Type II includes 2 isolates, Type III includes 6 isolates, Type IV includes 2 isolates, Type V includes 10 isolates, and 4 isolates are non-typeable.

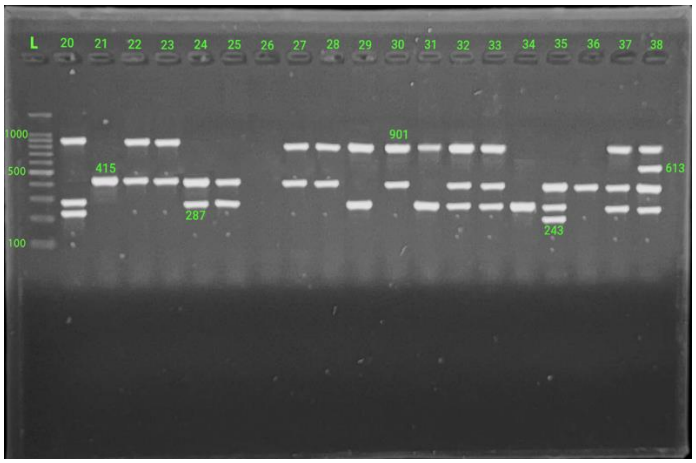

Figure 4: Multiplex PCR products of methicillin-resistant coagulase-negative Staphylococcus isolates. The distribution of SCCmec types among the isolates is as follows: Type I includes 1 isolate, Type II includes 11 isolates, Type III includes 2 isolates, Type IV includes 6 isolates, Type V includes 12 isolates, and 1 isolate is non-typeable.

Table 1: Primer Details for Detecting Staphylococcal Cassette Chromosome mec (SCCmec) Types.

Primer name	Sequence	Product size(bp)	Reference
Type I	F:GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	[34]
Type II	F: GATTACTTCAGAACCAGGTCAT R: TAAACTGTGTCACACGATCCAT	287	
Type III	F: CATTTGTGAAACACAGTACG R: GTTATTGAGACTCCTAAAGC	243	
Type VI	F: GAGGGATGGAGTGGATGAGATA R:GGTGAAGGACGATGAATGAGTAG	415	
Type V	F: CGAAGTAGTGATAGCCGCATAG R: GTATGGATGATCGGGCGTTAG	901	
mecA gene	F: TCCAGATTACAACTTCACCAGG R: CCACTTCATATCTTGTAACG	162	[35]

Table 2: Correlation Between Antibiotic Resistance Profile and Mobile Genetic Element Types in Methicillin-Resistant Coagulase-Negative Staphylococcus.

	SCCmec Type					
Antibiotics	**I n(2)**	**II n(19)**	**III n(8)**	**IV n(18)**	**V n(28)**	**Not contain N (6)**
Cefotaxime	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)

Augmentin	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)
Amoxicillin	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)
Oxacillin	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)
Ceftazidime	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)
Erythromycin	2 (100%)	19 (100%)	7 (87.5%)	17 (94.5%)	26 (92.8%)	6 (100%)
Piperacillin	2 (100%)	19 (100%)	8 (100%)	18 (100%)	28 (100%)	6 (100%)
Fusidic Acid	2p (100%)	15 (79%)	7 (87.5%)	18 (100%)	25 (89.2%)	5 (83.5%)
Tetracycline	2 (100%)	14 (74%)	6 (75%)	12 (66.6%)	20 (71.4%)	6 (100%)
Levofloxacin	2 (100%)	7 (37%)	6 (75%)	8 (44.4%)	19 (67.8%)	4 (66.6%)
Tobramycin	2 (100%)	16 (84%)	6 (75%)	13 (72%)	13 (72%)	5 (83.5%)
Gentamycin	2 (100%)	14 (74%)	4 (50%)	11 (61%)	25 (89.2%)	6 (100%)
Ciprofloxacin	2 (100%)	7 (37%)	6 (75%)	8 (44.4%)	20 (71.4%)	4 (66.6%)
Vancomycin	2 (100%)	17 (89%)	7 (87.5%)	14 (77.7%)	25 (89.2%)	5 (83.5%)
Clindamycin	2 (100%)	8 (42%)	4 (50%)	7 (38.8%)	13 (53.5%)	5 (83.5%)
Lincomycin	2 (100%)	12 (63%)	5 (62.5%)	8 (44.4%)	16 (57 %)	5 (83.5%)
Nitrofurantoin	1 (50%)	8 (42%)	0 (0%)	4 (22 %)	9 (32 %)	0 (0%)
Imipenem	1 (50%)	2 (10.5%)	1 (12.5)	1 (5.5%)	1 (3.5%)	4 (66.6%)
Chloramphenicol	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 3: Statistical Significance of Antibiotic Resistance Differences Among SCCmec Types.

Antibiotic Group	Representative Antibiotics	p-value	Key Observations
Beta-Lactams	Cefotaxime, Augmentin, Amoxicillin, Oxacillin, Ceftazidime, Piperacillin	N/A	100% resistance across all SCCmec types
Macrolides & Lincosamides	Erythromycin, Clindamycin, Lincomycin	$p < 0.05^{*}$	SCCmec II and IV exhibit lower resistance
Fluoroquinolones	Levofloxacin, Ciprofloxacin	$p < 0.001^{**}$	SCCmec II has the lowest resistance
Aminoglycosides	Tobramycin, Gentamycin	$p < 0.05$	SCCmec III shows the lowest resistance
Glycopeptides	Vancomycin	$p < 0.05$	SCCmec IV has lower resistance
Tetracyclines	Tetracycline	$p < 0.05$	SCCmec IV has the lowest resistance
Fusidic Acid	Fusidic Acid	$p < 0.05$	SCCmec III shows reduced resistance
Nitrofurans	Nitrofurantoin	$p < 0.001^{**}$	SCCmec III has 0% resistance
Carbapenems	Imipenem	$p < 0.001^{**}$	SCCmec V has the lowest resistance
Phenicols	Chloramphenicol	$p < 0.001^{**}$	Resistance is nearly absent across all SCCmec types

Table 4: Accession Number and Similarity Rate of Mobile Genetic Elements in Clinical Isolates from This Study Compared to Global Isolates in GenBank Using Sequencing Analysis.

No	Type	Name of isolate in GenBank	Accession Number (this study)	Accession Number (compared)in NCBI	Similarity (%)
1	**I**	*Staphylococcus haemolyticus* strain 12 *ccrB1*-like gene	PQ118407	CP035291.1	100%
2	**II**	*ccrB2*-like gene, partial sequence.	PQ151368	LR735432.1	100%
		ccrB2-like gene, partial sequence.	PQ151369	CP052055.1	100%
3	**III**	*ccrB3*-like gene, partial sequence.	PQ151370	CP043804.1	100%
		ccrB3-like gene, partial sequence.	PQ151371	CP035541.1	100%
4	**IV**	*Staphylococcus epidermidis* strain 4 ccrB2 gene	OR619697	CP120011.1	99.46%
		Staphylococcus epidermidis strain ST 1183 Ccrb2-like gene	PP990495	CP123738.1	100%
		Staphylococcus epidermidis strain ST35 *ccrB2*-like gene	PP990496	CP047851.1	100%
5	**V**	*Staphylococcus epidermidis* strain3 ST23 *ccrC*-like gene	PQ037176	CP043845.1	99.76%
		ccrC-like gene, partial sequence	PQ205317	CP025396.1	100%
6	***mecA***	*Staphylococcus epidermidis* strain 59 *mecA* gene	OR619696	CP133663.1	99.12%
		mecA gene, Partial sequence	PQ151372	CP043804.1	100%

4 Discussion

The findings of this study confirm that the analyzed isolates exhibit multidrug resistance (MDR), with 76% demonstrating resistance to six distinct classes of antibiotics: penicillins, cephalosporins, macrolides, glycopeptides, tetracyclines, and fluoroquinolones. An isolate is classified as MDR when it exhibits resistance to at least three antibiotic agents. Previous research has indicated that, due to this virulent trait—characterized by the ability to resist multiple antibiotics simultaneously—these bacteria are among the most challenging pathogens in the history of antimicrobial resistance[8]. MDR *Staphylococcus* can evade different agents through multiple resistance mechanisms, such as altering the target site, producing deactivating enzymes, employing efflux pumps, and decreasing the intracellular concentration of antibiotics [10]. All of these can arise firstly from prolonged and mistaken use of antibiotics by patients[11]. This study revealed that meropenem is a highly effective antibiotic against multidrug-resistant coagulase-

negative *Staphylococcus*. Meropenem acts on the cell wall, like other beta-lactam antibiotics, but differs in its high resistance to cephalosporinase or beta-lactamase enzymes [12]. However, chloramphenicol is the most effective antibiotic, with the highest efficacy rate. Chloramphenicol is a broad-spectrum antibiotic that exerts its antibacterial effects by inhibiting bacterial protein synthesis at the ribosomal level. Its primary mechanism of action involves binding to the 50S ribosomal subunit, specifically at the peptidyl transferase center. By doing so, it prevents the formation of peptide bonds between amino acids during translation, effectively halting polypeptide chain elongation. This inhibition disrupts bacterial protein synthesis, ultimately leading to bacteriostatic effects against most susceptible organisms[13].

The high susceptibilities recorded against these antibiotics could be due to their reserved use, primarily for resistant staphylococcal infections. Thus, last-resort antibiotics still retain high activity against CoNS and can be used for the empirical treatment of conditions such as suspected CoNS sepsis, even though resistance to these antibiotics is gradually increasing[14]. The results showed that a high percentage (100%) of isolates were methicillin-resistant *CoNS Staphylococcus*. There was a 100% agreement between phenotypic and genotypic confirmation of methicillin resistance in this study. This is mainly because the presence of the *mecA* gene encodes the protein PBP2A, which provides low affinity toward beta-lactam antibiotics [15].Methicillin resistance happened mainly because of a mutation in chromosome-encoded protein (penicillin-binding protein); bacteriophage transfers this mutation among CoNS isolates[16]. Methicillin resistance strains have always exhibited a wide number of virulence factors and consequently show multi-drug resistance through different mechanisms [17]. The *MRCoNS* strains showed 100% multidrug resistance, which is in agreement with [18].

The highest resistance rate of *CoNS* was observed toward amoxiclav, cefoxitin, methicillin, amoxicillin, and ceftriaxone. Methicillin resistance in isolates that lack the *mecA* gene may be mediated by other mechanisms, such as the possession of *mecC* and *mecB* genes[19]. The overproduction of β-lactamases, along with the emergence of methicillin resistance, has been documented in approximately 80% of *CoNS* species. This high prevalence significantly contributes to increased morbidity and mortality in hospital settings, as *CoNS* are major pathogens in healthcare-associated infections (HAIs)[14]. Resistance to macrolide antibiotics in staphylococcal strains is related to methicillin resistance[20]. The two primary resistance mechanisms developed by *S. aureus* against macrolides are the efflux of macrolides from the cell (mediated by the ATP-binding cassette family for ribosome protection) and the modification of bacterial ribosomes[21]. Resistance to glycopeptides was first recorded in the late 1980s with the emergence of vancomycin-resistant enterococci, attributed to the production of specific enzymes encoded by acquired operons responsible for eliminating natural peptidoglycan affinity precursors[22]. *SCCmec* type plays a crucial role in determining antibiotic resistance patterns, particularly among fluoroquinolones, macrolides, aminoglycosides, and carbapenems. Notably, *SCCmec* types II and III exhibit lower resistance to certain antibiotics, suggesting potential treatment options. While vancomycin and imipenem remain effective against *SCCmec* IV and V, emerging resistance warrants continuous monitoring. *SCCmec* type IV is frequently identified in *CoNS*, especially in *Staphylococcus epidermidis*, across both community and hospital environments. In one study of *S. epidermidis* hospital isolates, 36% were found to carry *SCCmec* type IV, 34% had type II, 28% had type III, and only 2% had type I *SCCmec*[23].

This study provides a comprehensive analysis of antibiotic resistance patterns among different *SCCmec* types, revealing significant variability across antibiotic classes. Beta-lactam antibiotics exhibited universal resistance (100%), highlighting their inefficacy against *SCCmec*-harboring strains. This finding is consistent with the presence of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a). Resistance to macrolides, lincosamides, fluoroquinolones, aminoglycosides, glycopeptides, and tetracyclines varied significantly ($p < 0.05–0.001$), with *SCCmec* II, III, and IV showing lower resistance in some cases, suggesting partial antibiotic efficacy against these strains. Among the tested antibiotics, nitrofurantoin and imipenem exhibited the most significant resistance differences ($p < 0.001$), with *SCCmec* III and V demonstrating the lowest resistance. This is likely due to the absence of specific resistance determinants or reduced selective pressure. This finding is clinically relevant, as imipenem remains a last-resort treatment for multidrug-resistant staphylococci.

Phenicol resistance was minimal, except in *SCCmec I*, indicating that resistance mechanisms against this class are not widespread, possibly due to lower historical exposure. These findings highlight the crucial role of *SCCmec* types in determining resistance patterns, emphasizing the potential for targeted therapeutic strategies and the need for continuous surveillance and molecular characterization of *SCCmec* elements to track emerging resistance trends and inform clinical decision-making as shown in Table 3. Another study on healthy individuals revealed that 57% of *S. epidermidis* isolates carried *SCCmec* type IV[24]. Additionally, other *SCCmec* types, such as type III, have been frequently reported in *S. epidermidis* isolates from both clinical and community settings[23], [25]. *SCCmec* can move between *S. epidermidis* and *S. aureus*, with *SCCmec* type IV being highly similar (98–99% identical) in both bacteria. Methicillin resistance is more common in *S. epidermidis* which is founded in hospitals (over 70%) than in *S. aureus*, suggesting that *S. epidermidis* possesses a wider variety of *SCCmec* types[23], [26].

There is evidence that the class C *mec* and *ccrC* complexes, which are part of *SCCmec*, originally came from *S. haemolyticus*. This includes *SCCmec* type V and other variations. This hypothesis is supported by the fact that the class C *mec* complexes in *MRSA* and methicillin-resistant *S. haemolyticus* are almost identical (99% similarity). A study by Barros et al. reported that 43% (24 isolates) of *S. haemolyticus* isolates were non-typeable, indicating significant genomic diversity within this species. Therefore, sequencing would be the most effective method to understand this diversity[27]. Genetic elements such as plasmids and transposons play a crucial role in the spread of antibiotic resistance genes

among staphylococci. These elements facilitate the transfer of resistance genes between bacteria, increasing their pathogenic potential. Insertion sequences are particularly effective at transferring resistance genes. *CoNS* strains can serve as reservoirs for resistance genes and transfer them to other bacteria, including *S. aureus*[28]. Antibiotic resistance in bacteria is a significant concern. To combat this issue, it is essential to study both pathogenic and non-pathogenic bacteria. Understanding the factors that drive antibiotic resistance can help develop effective prevention strategies [29]. Indeed CoNS have been found as repositories and sources of resistance features that spread throughout the Staphylococcaceae family [30],including resistance genes to last-resort antibiotics such as linezolid and daptomycin. CoNS from health care settings exhibit substantial resistance rates across geographic regions and infection locations[31]. Methicillin resistance in staphylococci is primarily caused by the *mecA* gene, which is located on mobile genetic elements known as *SCCmec*. These elements can facilitate the spread of resistance to other antibiotics, making *SCCmec* a key marker for multidrug-resistant staphylococci[30].

Most *SCCmec* types do not contain genes for antibiotic resistance, except for *mecA*. However, certain types, such as II and III, carry genes that confer the resistance to other antibiotics, including erythromycin, spectinomycin, kanamycin, tobramycin, bleomycin, tetracycline, and mercury. Additionally, types IX, X, and XI harbor genes that provide resistance to heavy metals such as cadmium, copper, and arsenate[32]. The discovery that *Staphylococcus haemolyticus* strain 12 contains a *ccrB1*-like gene with 100% similarity to a global isolate (*GenBank*: CP035291.1) highlights the potential worldwide spread of antibiotic resistance genes. The *ccrB1* gene, a key component of the *Staphylococcal Cassette Chromosome mec* (*SCCmec*), plays a crucial role in the horizontal transfer of methicillin resistance among staphylococcal species. Its complete sequence identity with a global isolate suggests that the gene is highly conserved, likely due to the selective pressure exerted by widespread antibiotic use. Similarly, the *ccrB2* gene in *Staphylococcus epidermidis* strain 4 shows 99.46% similarity to a global isolate (*GenBank*: CP120011.1), while strains *ST1183* and *ST35* exhibit 100% similarity to their respective global isolates. This finding suggests that these resistance determinants are widely conserved, potentially due to recent divergence or common ancestry. It further emphasizes the critical role of *SCCmec* elements in the global distribution of resistance genes.

The *ccrC*-like gene in *Staphylococcus epidermidis* strain *ST23*, with 99.76% similarity to a global isolate (*GenBank*: CP043845.1), further underscores the gene's conservation, likely due to its essential role in bacterial adaptability and resistance. Additionally, the *mecA* gene in *Staphylococcus epidermidis* strain 59 shows 99.12% similarity to a global isolate (*GenBank*: CP133663.1), as shown in Table 4. This indicates strong conservation of this crucial resistance gene, which encodes the penicillin-binding protein *PBP2a*, essential for methicillin resistance. Despite minor variations, the gene's core structure and function remain intact, emphasizing its role in the global spread of antibiotic resistance[33]. These findings collectively highlight the stability and potential dissemination of key resistance genes across different staphylococcal species and geographical regions. They suggest that the genetic elements responsible for antibiotic resistance are not only conserved but also likely spreading globally, underscoring the need for coordinated international efforts to monitor and control the spread of resistant strains.

Conclusion

In this study, *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* were identified as the most prevalent *Methicillin-Resistant Coagulase-Negative Staphylococci* (*MR-CoNS*) species. Both species exhibited high resistance to multiple antibiotics, particularly β-lactams, erythromycin, and fusidic acid. *SCCmec* typing revealed significant genetic diversity, with *SCCmec* V being the most common, while *SCCmec* I displayed the highest resistance across almost all tested antibiotics. Notably, chloramphenicol and imipenem showed the least resistance, suggesting their potential effectiveness against these *MR-CoNS* isolates. Sequencing analysis revealed a high genetic similarity between the studied isolates and global counterparts, underscoring the widespread distribution and conservation of resistance genes. These findings highlight the critical need for ongoing surveillance and tailored antibiotic strategies to combat *MR-CoNS* infections.

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Contributions

Methodology & formal analysis, M.O.I; Supervision & Review and editing, H.K.B, A.H.A.

Competing Interests

The authors declare no conflicts of interest**.**

Approval

The current study originates from a medical student's thesis and has received approval from the Ethical Approval Committee of the University of Anbar, Iraq.

Consent for Publication

Not applicable.

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