

# Antibiotic Susceptibility Patterns of Biofilm-producing Nosocomial Non-coagulase *Staphylococcus* Isolates from Clinical Sources in Iraq



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## Abstract:

**Introduction:** Coagulase-negative staphylococci (CoNS), like *S. epidermidis* and *S. haemolyticus*, are skin commensals that can cause infections. Their biofilms increase resistance and persistence. This study assessed biofilm formation, antibiotic resistance, and genetic diversity of local CoNS using MLST to aid regional surveillance.

**Methods:** In this study, samples were collected, and the species were identified using the VITEK 2 Compact system. A Kirby-Bauer disc diffusion test was carried out to detect antimicrobial susceptibility, and the biofilm production was tested with a microtiter plate. Multilocus sequence typing (MLST) was performed, followed by data processing using the Oxford scheme to categorize the isolates.

**Results:** Among 150 clinical samples, 13.3% were *S. epidermidis* and 20% *S. haemolyticus*. Coagulase-negative staphylococci (CoNS) showed 100% resistance to amoxiclav, ceftazidime, methicillin, amoxicillin, and ceftriaxone. High resistance (66–96%) was seen for several antibiotics, with 76% classified as multidrug-resistant (MDR). All CoNS formed biofilms (58% strong). MLST revealed diverse sequence types (STs), including newly reported ST23 and ST59 for *S. epidermidis*, and ST24, ST35, and ST109 for *S. haemolyticus* in Iraq.

**Discussion:** Multidrug-resistant CoNS isolates showed high biofilm formation and *mecA* gene presence, indicating significant clinical infection and resistance challenges.

**Conclusion:** This study provides valuable data on the prevalence, antibiotic resistance, and genetic diversity of *S. epidermidis* and *S. haemolyticus* strains in Anbar, Iraq. It also reports several novel MLST sequence types for the first time in the region, including unique strain IDs for both species, contributing to improved local epidemiological knowledge and surveillance efforts.

**Keywords:** *S. haemolyticus*, *S. epidermidis*, MLST, MDR, Biofilm.

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## 1. INTRODUCTION

Coagulase-negative *staphylococci* (CoNS), including *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, are commonly present on the skin and mucous membranes of humans and animals [1].

They are acknowledged as opportunistic pathogens, with *Staphylococcus epidermidis* being the primary coagulase-negative *Staphylococcus* (CoNS) responsible for healthcare-associated infections (HAIs) [2]. HAIs, previously known as nosocomial or hospital-acquired infections, are infections acquired by patients during medical treatment in healthcare settings. They are a major contributor to illness and death among hospitalized patients [3]. According to the World Health Organization (WHO), approximately 7% of hospitalized patients in developed countries and 10% in developing countries are estimated to acquire at least one healthcare-associated infection (HAI) at any given time [4]. CoNS are primarily recognized as commensals on human and animal bodies, but they can also act as opportunistic pathogens. The composition of commensal skin flora varies by body site, reflecting the differing environmental conditions at each location [5]. It is noteworthy that biofilm-associated infections resist responding to different antimicrobial agents, the immune system, as well as harsh conditions; thus, biofilm formation is an important characteristic in the progression of pathogenicity. Biofilm-forming isolates contribute to infections associated with catheters and other medical equipment [6]. Biofilm-producing *S. haemolyticus* is a major cause of bacteremia, particularly in catheter-associated and nosocomial infections. Biofilm formation by *S. haemolyticus* is a complex process that is further enhanced in the presence of antimicrobial agents [7]. 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 [8]. The most commonly isolated MR-CoNS species in hospitals are *S. epidermidis* and *S. haemolyticus* [9].

MLST is a molecular typing technique employed for bacteria identification and classification. It relies on analyzing DNA sequences from seven highly conserved housekeeping genes essential for cellular function [10]. These gene sequences are amplified via PCR and then compared against a reference database of known MLST types, allowing for the identification and assignment of the isolate to an MLST type. This classification offers insights into the bacterium's genetic lineage and potential pathogenicity [11]. This study aimed to identify prevalent local isolates and distinguish infectious pathogen strains using MLST to enhance epidemiological surveillance and strain source determination. Additionally, it investigated biofilm formation and antibiotic susceptibility patterns in *Staphylococcus* isolates. Given its role as a significant human pathogen, particularly in immunocompromised individuals, long-term hospitalized patients, and those with severe illnesses, understanding its genetic diversity

and resistance profiles is crucial for effective infection control.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial Sampling and Identification

Between December 2022 and June 2023, a total of 150 clinical samples were collected, including wounds, burns, urine, ear swabs, and blood samples. *Staphylococcus* isolates were identified based on their growth on blood agar and mannitol salt agar. Characterization was carried out using conventional microbiological methods, including colonial morphology, Gram staining, and biochemical tests. Final identification was conducted using the VITEK 2 Compact system (bioMérieux, France), following the bacterial identification protocol cited previously [10].

### 2.2. Detection of Antibiotic Resistance Profile

The Kirby-Bauer disc diffusion method used 19 antibiotic discs (“Oxoid, UK”). The antibiotic susceptibility of the isolates was tested against a range of antibiotics, including amoxiclav, cefoxitin, methicillin, amoxicillin, ceftriaxone, piperacillin, ceftazidime, fusidic acid, tobramycin, tetracycline, gentamicin, azithromycin, vancomycin, clindamycin, lincomycin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, rifampicin, nitrofurantoin, meropenem, and chloramphenicol.

### 2.3. Biofilm Formation

Biofilm production was assessed using the microtiter plate method, as described by Yousefi *et al.* (2016).

### 2.4. Molecular Screening of *mecA* Gene

Bacterial DNA was extracted using the Promega DNA Mini Kit provided by Promega, USA. DNA was extracted from the bacteria, and its purity was measured. A specific gene, *mecA*, was then tested for forward “(5'-TCCAGATTACAACCTTCACCAGG-3')” and reverse “(5'-CCACTTCATATCTTGTAACG-3')” primers, following the method described by Ghaznavi-Rad *et al.* 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 (Biotek, China). A 100 bp DNA ladder (Vivantis, Malaysia) served as a marker. The gel was visualized under UV light and imaged using a gel imager (Major Science, USA) [12].

### 2.5. Multilocus Sequence Typing

All strains were analyzed by the MLST protocol using primers listed in Tables 1 and 2. All PCR reactions were carried out in Applied Biosystems 2720 Thermal Cycler (USA).

**Table 1. Genes and primer sequence for *Staphylococcus haemolyticus* used in the MLST scheme [13].**

Gene Loci	Primer Sequence (5-3)	Amplicon Size
Arc <sup>a</sup>	F AGTGACTCAAGTTGAA R AATCTTACCATCTAGG	600
SH 1200 <sup>b</sup>	F CGGTAATGTAACACACGCAGT R TCTTCCTAGTAGCTGACCAG	540
HemH <sup>c</sup>	F CTGATCGTCAAGCTGAAGCAT R GTACCTGTGTGACCCTCAGA	500
leuB <sup>d</sup>	F AGCCATAGATTCGCATGGTGT R CCTAATGAACCTGGAATGGTAG	650
SH 1431 <sup>e</sup>	F TCAGACCAATTCCCAACC R CTTAGCGTCACGATGGTCG	600
CfxE <sup>f</sup>	F GAAGCACAAATTGATGGTCTGC R TCTGCCCAATTATCAACACA	500
Ribose ABC	F GAGACGATTCAGCTAAGCAA R CGCCTTTCATTAGGCCATTA	650

**Table 2. Genes and primer sequence for *Staphylococcus epidermidis* used in the MLST scheme [14].**

Gene	Primer sequence (5'-3')	Amplicon (bp)
arcC	F TGTGATGAGCAGCTACCGTTAG R TCCAAGTAAACCCATCGGTCTG	508
aroE	F CATTGGATTACCTCTTTGTTTCAGC "R CAAGCGAAATCTGTTGGGG"	459
gtr	"F CAGCCAAITCTTTTATGACTTTT" "R GTGATTAAAGGTATTGATTGAAT"	508
mutS	F GATATAAGAATAAGGGTTGTGAA R GTAATCGTCTCAGTTATCATGTT	608
pyr	F GTTACTAATACTTTTGCTGTGTTT R GTAGAATGTAAAGAGACTAAATGAA	851
Tpi	F ATCCAATTAGACGCTTTAGTAAC R TTAATGATGCGCCACCTACA	592
yqiL	F CACGCATAGTATTAGCTGAAG R CTAATGCCTTCATCTTGAGAAATAA	658

## 2.6. Statistical Analysis

Statistical analyses were performed using a chi-square test to determine whether the distribution of resistance percentages varies for each antibiotic, along with the relationship between the source of isolate and biofilm formation.

## 2.7. Ethical Approval

The study obtained approval from the Ethics Committee of Al Anbar Medical Research University (approval number 23, December 5<sup>th</sup>, 2022). All participants provided consent, and no individuals under 16 years old were involved.

## 3. RESULTS AND DISCUSSION

### 3.1. Phenotypic Identification of Clinical Isolates

In the period from December 2022 to June 2023, 150 samples were collected from clinical sources, including patients of both sexes from different teaching hospitals and outpatients in Anbar/Iraq. All these samples were

grown on mannitol salt agar and blood agar. The bacterial isolates included were 13.3% (n=20) *S. epidermidis*, 20% (n=30) *S. haemolyticus*, 23.3% (n=35) coagulase-positive staphylococci, 26% (n=39) Gram-negative bacteria, and 17.3% (n=26) showed no growth. Higher prevalence rates of methicillin-resistant coagulase-negative staphylococci (MRCoNS) have been reported in South Africa, reporting 86% in one study [14] and 100% in another study [15]. Among CoNS associated with infections, *S. epidermidis* and *S. haemolyticus* are recognized as significant etiological agents causing nosocomial infections [16]. Notably, *S. epidermidis* is the most frequently isolated staphylococcal species in humans and is considered the most clinically relevant CoNS species [17]. The highest percentage of coagulase-negative *Staphylococcus* (CoNS) was isolated from wound samples (36%), followed by burn samples (20%) and urine samples (18%). The lowest percentages were observed in ear swabs and blood samples, with 14% and 12%, respectively.

### 3.2. Antibiotic Sensitivity of Coagulase-Negative Staphylococcus

The antibiotic susceptibility testing of coagulase-negative *Staphylococcus* (CoNS) isolates showed the highest resistance rates to amoxiclav, cefoxitin, methicillin, amoxicillin, and ceftriaxone (100%). The isolates also exhibited high resistance to piperacillin (96%), ceftazidime (96%), fusidic acid (76%), tobramycin (68%), tetracycline (68%), gentamicin (66%), and azithromycin (66%). Resistance to vancomycin was observed in 52% of the isolates. Resistance levels were low against clindamycin (46%), lincomycin (42%), ciprofloxacin (42%), levofloxacin (40%), trimethoprim-sulfamethoxazole (38%), rifampicin (38%), and nitrofurantoin (18%). In comparison, the lowest resistance was observed against meropenem (10%) and chloramphenicol (2%). The results also confirmed that the study isolates were considered multidrug-resistant (MDR), with 76% showing resistance to six classes of antibiotics: penicillin, cephalosporins, macrolides, glycopeptides, tetracyclines, and fluoroquinolones.

An isolate is considered MDR when it shows resistance to at least three classes of antibiotic agents [18]. This virulent characteristic, *i.e.*, its ability to resist multiple classes of antibiotics simultaneously, makes the bacterium one of the most intractable pathogens in the history of antibiotics [19]. MDR *Staphylococcus* can resist various agents through multiple mechanisms, such as altering the target site, producing deactivating enzymes, efflux pumping, and decreasing the intracellular concentration of antibiotics [20]. These mechanisms often arise due to prolonged or improper antibiotic use by patients [21]. 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 [22].

However, chloramphenicol is the most effective antibiotic, exhibiting the highest efficacy. It 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, to prevent 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 [23]. The high susceptibilities recorded against these antibiotics could be due to the reserved use of those antibiotics, mainly for resistant staphylococcal infections. Thus, last-resort antibiotics still retain high activity against coagulase-negative *Staphylococcus* (CoNS) and may be used for empirical treatment of conditions, such as suspected CoNS sepsis, even though resistance to these antibiotics is gradually increasing [24]. The results showed that all isolates (100%) were methicillin-resistant coagulase-negative *Staphylococcus*. There was a complete concordance between phenotypic and genotypic results in confirming methicillin resistance in this study. This is primarily due to the presence of the *mecA* gene, which encodes the PBP2a protein that has a low affinity for beta-lactam antibiotics [25].

### 3.3. Biofilm Formation

The results of the microtiter plate (MTP) assay revealed that all the isolates demonstrated a positive ability for biofilm formation. Based on their biofilm-producing capacity, the isolates were classified into three categories: 58% (n=29) exhibited strong biofilm formation, 30% (n=15) displayed moderate biofilm production, and 12% (n=6) showed weak biofilm formation. These findings highlight the varying degrees of biofilm production among the isolates, which may have implications for their pathogenicity and persistence in clinical settings. Statistical analysis was performed to determine which clinical source was associated with the highest level of biofilm production and whether the observed differences were statistically significant. The results indicated that coagulase-negative *Staphylococcus* isolates from urine and wound sources exhibited significantly higher biofilm production compared to isolates from blood and ear swabs. Although biofilm production in burn isolates was higher than in those from blood and ear swab samples, the difference was not statistically significant (Table 3).

Isolates of *Staphylococcus haemolyticus* from urine and wound samples showed significantly higher biofilm production than isolates from blood and ear swabs. Burn isolates demonstrated elevated biofilm levels but without statistical significance. This suggests that specific clinical

**Table 3. Presents a statistically significant difference in biofilm production among different sources ( $p < 0.05$ ).**

Comparison	P-value	Significance ( $p < 0.05$ )
Urine vs. Blood	< 0.01	Significant
Wound vs. Blood	< 0.01	Significant
Burn vs. Blood	0.07	Not Significant
Ear Swab vs. Blood	0.08	Not Significant
Urine vs. Wound	0.90	Not Significant
Urine vs. Burn	0.04	Significant



environments may favor biofilm formation due to nutrient availability or local stress factors. Another study indicated that biofilm production was most prevalent in blood samples (27.3%), followed by pus swabs (12.5%) and pus aspirates (16.7%). Biofilm development is strongly associated with medical device-related infections, including contaminated implants, urinary catheters, and prosthetic valves [26]. Biofilm is an extracellular polysaccharide layer that facilitates bacterial attachment to surfaces and medical devices. *S. haemolyticus* isolates capable of forming biofilms contribute to infections associated with catheters and other medical devices [7]. *S. epidermidis*-producing biofilms can lead to bacteremia, especially in cases linked to catheter-associated and nosocomial infections. Biofilm formation by *S. haemolyticus* is a complex process that becomes more pronounced in the presence of antimicrobial agents [27].

### 3.4. Molecular Screening of *mecA* Gene

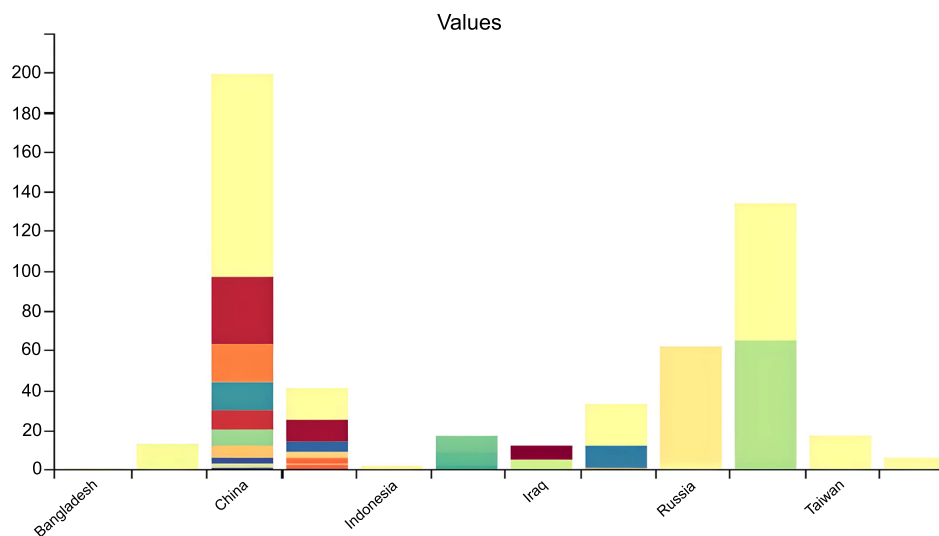
The results demonstrated that all the isolates were 100% positive for the detection of the *mecA* gene in methicillin-resistant coagulase-negative *Staphylococcus* isolates by using Uniplex PCR. Methicillin resistance occurs mainly because of a mutation in chromosome-encoded protein (penicillin-binding protein); bacteriophage transfers this mutation among CoNS isolates [28]. Methicillin-resistant strains have always exhibited a wide number of virulence factors and consequently show multi-drug resistance through different mechanisms [29]. In this study, the MRCoNS strains showed 100% multi-drug resistance, which is in agreement with the findings of a previous study [30].

The highest resistance rate of CoNS toward amoxiclav, cefoxitin, methicillin, amoxicillin, and ceftriaxone was observed. Methicillin resistance in isolates that lack the *mecA* gene may be mediated by other mechanisms of

methicillin resistance, such as the presence of *mecC* and *mecB* genes [31]. The overproduction of  $\beta$ -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) [24].

### 3.5. MLST Analysis

The molecular analysis identified five distinct sequence types (STs) of *Staphylococcus epidermidis*, demonstrating a high level of genotypic diversity. Notably, all isolates were recorded in Iraq for the first time, as presented in Table 4 and Fig. (1). ST89 was initially reported in Denmark in 1997 with ID 40259, with a global frequency of 27 isolates. In the current study, this ST has been identified in Iraq with ID 46590. Similarly, ST23 was first recorded in Mexico in 1996 with ID 40147 and has now been documented in Iraq with ID 46588. ST59 was originally detected in South Korea with ID 41234, and in this study, it has been identified in Iraq with ID 46587. This sequence type exhibited the highest frequency in this study, with a global occurrence of 101 isolates. Additionally, ST1183 was found to have the lowest global frequency, with only three recorded strains. These findings highlight the genetic diversity of *S. epidermidis* isolates and emphasize the importance of continued molecular surveillance to understand the epidemiology and distribution of this opportunistic pathogen. Moreover, there is a need for targeted public health measures and further research in Iraq. The *Staphylococcus haemolyticus* isolates analyzed in this study provide valuable insights into their global distribution and epidemiological significance. Sequence type (ST) 24 was first recorded in India in 2010 with ID 55, while in the current study, this isolate was identified in Iraq with ID 240. Similarly, ST35



**Fig. (1).** Shows the percentage distribution of *Staphylococcus epidermidis* isolates across Asia, highlighting regional variations. Notably, Iraq shows a significant proportion, possibly due to hospital-acquired infections, antimicrobial resistance, or diagnostic differences.

was initially reported in India in 2016 with ID 70 and has now been detected in Iraq with ID 241. ST109 was first identified in China in 2019 with ID 151, and in the present study, it has been recorded in Iraq with ID 242, with a frequency of two isolates. ST173 was initially reported in Burma in 2023 with ID 238 and has now been recorded in Iraq with ID 243. Likewise, ST146 was first documented in Thailand in 2021 with ID 197, and in the current study,

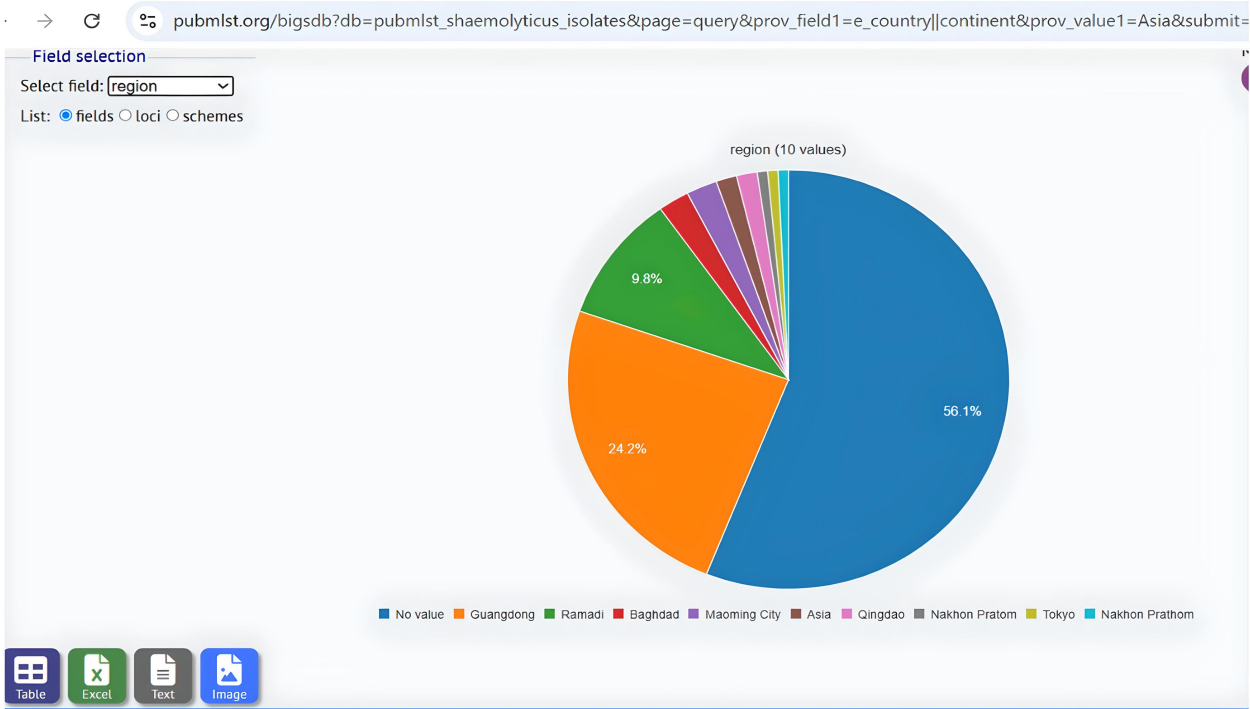
this isolate has been detected in Iraq with ID 244. Other details of ST under study are listed in Table 4 and Fig. (2).

Alleles and sequence types (STs) were identified using the *S. epidermidis*-specific MLST database ([https://pubmlst.org/bigsdb?db=pubmlst\\_sepidermidis\\_isolates&page=profiles&scheme\\_id=4](https://pubmlst.org/bigsdb?db=pubmlst_sepidermidis_isolates&page=profiles&scheme_id=4)) and for *S. haemolyticus* isolate, using its corresponding MLST database [https://pubmlst.org/bigsdb?db=pubmlst\\_shaemolyticus\\_isolates&page=profiles&scheme\\_id=1](https://pubmlst.org/bigsdb?db=pubmlst_shaemolyticus_isolates&page=profiles&scheme_id=1)).

**Table 4. Presents data on *Staphylococcus epidermidis* (1-5) and *Staphylococcus haemolyticus* (6-10) isolates, detailing their sequence types (STs), ID profiles, geographic origin, isolation sources, year of first global identification, study-specific identification numbers, and frequency of occurrence.**

Isolates	ST	Methicillin Susceptibility	ID Profile	Isolation Area First Time	Source of Isolation	First Isolation Year in the World	Special ID for the Current Study	Frequency
1.	89	*MRSE	40259	Denmark	blood	1997	46590	27 isolates
2.	23	MRSE	40147	Mexico	Blood	1996	46588	60 isolates
3.	59	MRSE	41234	South Korea	Wound swab	2000	46587	101 isolates
4.	1183	MRSE	44665	South Africa	CSF		46591	3 isolates
5.	35	MRSE	40165	Portugal	Nasal swab	2000	46592	22 isolates
6.	24	*MRSH	55	India	Wound	2010	240	2 isolates
7.	35	MRSH	70	India	Wound	2016	241	2 isolates
8.	109	MRSH	151	China	UTI	2019	242	2 isolates
9.	173	MRSH	238	Burma	Wound	2023	243	2 isolates
10.	146	MRSH	197	Thailand	Wound	2021	244	2 isolates

\*MSRE: Methicillin Resistance *S. epidermidis*, \*MSRH: Methicillin Resistance *S. haemolyticus*



**Fig. (2).** Shows the percentage distribution of *Staphylococcus haemolyticus* isolates across Asia, highlighting their prevalence in various countries. The green section represents the isolates recorded in Iraq, specifically in Ramadi, from the current study. This image presents a comparative analysis of the geographical spread of *S. haemolyticus*, emphasizing the newly identified isolates in Iraq and their contribution to the regional distribution.

The detection of various sequence types (STs) in Iraq suggests possible intercontinental transmission or convergent evolution, potentially driven by selective pressures in hospital environments. ST89 (from Denmark), ST23 (from Mexico), and the rare ST1183 indicate global dissemination and the importance of monitoring uncommon STs for antimicrobial resistance. For *Staphylococcus haemolyticus*, five distinct STs were identified, namely ST24 and ST35 (from India), ST109 (from China), ST173 (from Burma), and ST146 (from Thailand), indicating regional spread within Asia and broader international movement. This underscores the genetic diversity and global distribution of these pathogens. The emergence of these sequence types in Iraq may result from globalization, hospital transmission, and antimicrobial selective pressures [32]. The presence of globally distributed STs in *S. epidermidis* and *S. haemolyticus* suggests that healthcare-associated infections (HAIs) influence their population structure. As major opportunistic pathogens linked to bloodstream and prosthetic device infections with multidrug resistance, molecular surveillance is essential [33]. The detection of sequence types with varying global frequencies in Iraq highlights the need for continuous genetic monitoring to track bacterial evolution, antimicrobial resistance, and transmission pathways. Using MLST databases enabled precise classification and epidemiological assessment. Identifying both novel and known *S. epidermidis* and *S. haemolyticus* STs reveals their genetic diversity and clinical significance. Ongoing molecular surveillance is crucial to understand their distribution, resistance profiles, and pathogenic potential, supporting effective infection control strategies [34]. Another study indicated that MLST analysis of *S. epidermidis* identifies high-risk clones linked to nosocomial infections, tracks antibiotic-resistant strains, and guides infection control and antibiotic therapy. It enhances understanding of *S. epidermidis* epidemiology, supporting strategies to prevent and manage infections [35].

## CONCLUSION

This study reveals high methicillin resistance, multidrug resistance (MDR), and strong biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from Anbar, Iraq. The identification of diverse sequence types (STs), reported for the first time in Iraq, highlights their genetic diversity and potential for intercontinental transmission. The presence of the *mecA* gene in all MRCoNS underscores the antimicrobial resistance threat. These findings emphasize the need for continuous molecular surveillance to track bacterial evolution and resistance, supporting effective infection control strategies and antibiotic stewardship in healthcare settings.

## AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the paper as follows: H.K.B.: Study Concept or Design; A.H.A.: Data Curation; All authors reviewed the results and approved the final version of the manuscript.

## LIST OF ABBREVIATIONS

MLST	=	Multilocus sequence typing
CoNS	=	Coagulase-negative staphylococci
HAIs	=	Healthcare-associated infections
MTP	=	Microtiter plate

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study obtained approval from the Ethics Committee of Al Anbar Medical Research University, Iraq, (approval number 23, December 5<sup>th</sup>, 2022).

## HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

## CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

## REFERENCES

- [1] Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev 2014; 27(4): 870-926. <http://dx.doi.org/10.1128/CMR.00109-13> PMID: 25278577
- [2] Al-kubaisy SHM, Khalaf BH, Khaleef EL. Nosocomial infections: Types and prevalence. J Univ Anbar Pure Sci 2022; 16(2): 1-6.
- [3] Marsteller JA, Hsu YJ, Weeks K. Evaluating the impact of mandatory public reporting on participation and performance in a program to reduce central line-associated bloodstream infections: Evidence from a national patient safety collaborative. Am J Infect Control 2014; 42(10): S209-15. <http://dx.doi.org/10.1016/j.ajic.2014.06.001> PMID: 25239712
- [4] Katoziyan A, Imani Fooladi AA, Taheri RA, Vatanpour S. "Multi-drug resistance of staphylococcus aureus strains in baqiyatallah hospital: A primary step towards digital health biomonitoring systems," Iran. Iran J Pharm Res 2020; 19(3): 321-8. PMID: 33680033
- [5] Myles IA, Moore IN, Castillo CR, Datta SK. Differing virulence of healthy skin commensals in mouse models of infection. Front Cell Infect Microbiol 2019; 8: 451. <http://dx.doi.org/10.3389/fcimb.2018.00451> PMID: 30719426
- [6] Flahaut S, Vinogradov E, Kelley KA, Brennan S, Hiramatsu K, Lee JC. Structural and biological characterization of a capsular polysaccharide produced by *Staphylococcus haemolyticus*. J Bacteriol 2008; 190(5): 1649-57. <http://dx.doi.org/10.1128/JB.01648-07> PMID: 18165309

- [7] Pereira-Ribeiro PMA, Sued-Karam BR, Faria YV, *et al.* Influence of antibiotics on biofilm formation by different clones of nosocomial *Staphylococcus haemolyticus*. *Future Microbiol* 2019; 14(9): 789-99.  
<http://dx.doi.org/10.2217/fmb-2018-0230> PMID: 31271299
- [8] Wojtyczka R, Orlewska K, Kępa M, *et al.* Biofilm formation and antimicrobial susceptibility of *Staphylococcus epidermidis* strains from a hospital environment. *Int J Environ Res Public Health* 2014; 11(5): 4619-33.  
<http://dx.doi.org/10.3390/ijerph110504619> PMID: 24776724
- [9] Mehdinejad M, Sheikh AF, Jolodar A. Study of methicillin resistance in *Staphylococcus aureus* and species of coagulase negative staphylococci isolated from various clinical specimens. *Pak J Med Sci* 2008; 24(5): 719-24.
- [10] Lahij HF, Almeani SAL. Multidrug-resistant clinical *K. pneumoniae* ST16, ST218, and ST283 and emergence of pandrug-resistant KPC-positive ST6434/K2 lineage in Iraq. *Braz J Microbiol* 2024; 55(1): 375-82.  
<http://dx.doi.org/10.1007/s42770-023-01205-w> PMID: 38091237
- [11] Weng PL, Ramli R, Shamsudin MN, Cheah Y-K, Hamat RA. High genetic diversity of *Enterococcus faecium* and *Enterococcus faecalis* clinical isolates by pulsed-field gel electrophoresis and multilocus sequence typing from a hospital in Malaysia. *Biomed Res Int* 2013; 2013: 938937.  
<http://dx.doi.org/10.1155/2013/938937>
- [12] Martínez-Santos VI, Torres-Añorve DA, Echániz-Aviles G, Parra-Rojas I, Ramírez-Peralta A, Castro-Alarcón N. Characterization of *Staphylococcus epidermidis* clinical isolates from hospitalized patients with bloodstream infection obtained in two time periods. *PeerJ* 2022; 10: 14030.  
<http://dx.doi.org/10.7717/peerj.14030> PMID: 36213498
- [13] Maiden M C J, *et al.* MLST revisited: The gene-by-gene approach to bacterial genomics. *Nat Rev Microbiol* 2013; 11(10): 728-36.  
<http://dx.doi.org/10.1038/nrmicro3093>
- [14] Ballot DE, Nana T, Sriruttan C, Cooper PA. Bacterial bloodstream infections in neonates in a developing country. *ISRN Pediatr* 2012; 2012(1): 1-6.  
<http://dx.doi.org/10.5402/2012/508512> PMID: 22919509
- [15] Ehlers MM, Strasheim W, Lowe M, Ueckermann V, Kock MM. Molecular epidemiology of *Staphylococcus epidermidis* implicated in catheter-related bloodstream infections at an Academic Hospital in Pretoria, South Africa. *Front Microbiol* 2018; 9: 417.  
<http://dx.doi.org/10.3389/fmicb.2018.00417> PMID: 29563910
- [16] Gómez-Sanz E, Ceballos S, Ruiz-Ripa L, Zarazaga M, Torres C. Clonally diverse methicillin and multidrug resistant coagulase negative staphylococci are ubiquitous and pose transfer ability between pets and their owners. *Front Microbiol* 2019; 10: 485.  
<http://dx.doi.org/10.3389/fmicb.2019.00485> PMID: 30972035
- [17] Namvar AE, Bastarahang S, Abbasi N, *et al.* Clinical characteristics of *Staphylococcus epidermidis*: A systematic review. *GMS Hyg Infect Control* 2014; 9(3): Doc23.  
<http://dx.doi.org/10.3205/dgkh000243> PMID: 25285267
- [18] Turner NA, Sharma-Kuinkel BK, Maskarinec SA, *et al.* Methicillin-resistant *Staphylococcus aureus*: An overview of basic and clinical research. *Nat Rev Microbiol* 2019; 17(4): 203-18.  
<http://dx.doi.org/10.1038/s41579-018-0147-4> PMID: 30737488
- [19] Suganya T, Packiavathy IASV, Aseervatham GSB, *et al.* Tackling multiple-drug-resistant bacteria with conventional and complex phytochemicals. *Front Cell Infect Microbiol* 2022; 12: 883839.  
<http://dx.doi.org/10.3389/fcimb.2022.883839> PMID: 35846771
- [20] Rajput P, Nahar KS, Rahman KM. Evaluation of antibiotic resistance mechanisms in gram-positive bacteria. *Antibiotics* 2024; 13(12): 1197.  
<http://dx.doi.org/10.3390/antibiotics13121197> PMID: 39766587
- [21] Mukherjee R, Priyadarshini A, Pandey RP, Raj VS. Antimicrobial resistance in *Staphylococcus aureus*. *Staphylococcus Aureus*. London, UK: InTechOpen 2021; pp. 1-7.  
<http://dx.doi.org/10.5772/intechopen.96888>
- [22] Alfei S, Schito AM.  $\beta$ -lactam antibiotics and  $\beta$ -lactamase enzymes inhibitors, part 2: Our limited resources. *Pharmaceutics* 2022; 15(4): 476.  
<http://dx.doi.org/10.3390/ph15040476> PMID: 35455473
- [23] Yu T, Zeng F. Chloramphenicol interferes with 50s ribosomal subunit maturation via direct and indirect mechanisms. *Biomolecules* 2024; 14(10): 1225.  
<http://dx.doi.org/10.3390/biom14101225> PMID: 39456158
- [24] Asante J, Amoako DG, Abia ALK, *et al.* Review of clinically and epidemiologically relevant coagulase-negative staphylococci in Africa. *Microb Drug Resist* 2020; 26(8): 951-70.  
<http://dx.doi.org/10.1089/mdr.2019.0381> PMID: 32043916
- [25] Lade H, Kim JS. Molecular determinants of  $\beta$ -lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA): An updated review. *Antibiotics* 2023; 12(9): 1362.  
<http://dx.doi.org/10.3390/antibiotics12091362> PMID: 37760659
- [26] Dilip DS, Menon AR. Speciation, detection of virulence factors and antibiotic susceptibility of coagulase negative staphylococci. *Int J Sci Res Dent Med Sci* 2021; 3(3): 122-32.  
<http://dx.doi.org/10.30485/ijdsrds.2021.289102.1170>
- [27] Dier-Pereira AP, *et al.* "Methicillin-resistant staphylococcus haemolyticus displaying reduced susceptibility to vancomycin and high biofilm-forming ability." *Infect. Disord. TargetsDisorders* 2021; 21(7): e160921191517.  
<http://dx.doi.org/10.2174/1871526521666210217151807>
- [28] Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev* 2018; 31(4): e00020-18.  
<http://dx.doi.org/10.1128/CMR.00020-18> PMID: 30209034
- [29] Algammal AM, Hetta HF, Elkesh A, *et al.* Methicillin-Resistant *Staphylococcus aureus* (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infect Drug Resist* 2020; 13: 3255-65.  
<http://dx.doi.org/10.2147/IDR.S272733> PMID: 33061472
- [30] Adhikari P, Basyal D, Rai JR, *et al.* Prevalence, antimicrobial susceptibility pattern and multidrug resistance of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples at a tertiary care teaching hospital: An observational, cross-sectional study from the Himalayan country, Nepal. *BMJ Open* 2023; 13(5): 067384.  
<http://dx.doi.org/10.1136/bmjopen-2022-067384> PMID: 37164471
- [31] Dhaouadi S, Soufi L, Campanile F, *et al.* Prevalence of methicillin-resistant and -susceptible coagulase-negative staphylococci with the first detection of the *mecC* gene among cows, humans and manure in Tunisia. *Int J Antimicrob Agents* 2020; 55(1): 105826.  
<http://dx.doi.org/10.1016/j.ijantimicag.2019.10.007> PMID: 31634550
- [32] Berndtson AE. Increasing globalization and the movement of antimicrobial resistance between countries. *Surg Infect* 2020; 21(7): 579-85.  
<http://dx.doi.org/10.1089/sur.2020.145> PMID: 32434446
- [33] Chong J. Molecular epidemiology and clinical impact of heteroresistant vancomycin-intermediate coagulase negative staphylococci in the NICU. Canada: McGill University 2016; pp. 1-8.
- [34] Ogunlaja A, Oginni O. Molecular epidemiology and phylodynamics of emerging and re-emerging viral infections: Implications for surveillance and control. *Inter J Res Publicat Rev* 2022; 6(2): 2924-41.  
<http://dx.doi.org/10.55248/gengpi.6.0225.0937>
- [35] Jamet A, Guglielmini J, Brancotte B, *et al.* High-resolution typing of *Staphylococcus epidermidis* based on core genome multilocus sequence typing to investigate the hospital spread of multidrug-resistant clones. *J Clin Microbiol* 2021; 59(3): e02454-20.  
<http://dx.doi.org/10.1128/JCM.02454-20> PMID: 33328176