Challenge of frequency of blaZ gene among *Staphylococcus epidermidis* harboring mecA gene isolated from clinical specimens in Al-Basrah province, Iraq

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Abstract
Background: Rapidly identifying *Staphylococcus epidermidis* isolates from various types of coagulase-negative staphylococci (CoNS) is essential, but identifying resistant agents can also significantly enhance existing diagnostic and treatment approaches.
Aim of study: Determine resistance to methicillin and antibiotic susceptibility pattern of β-lactam drugs and detect *mecA* and *blaZ* among *S.epidermidis* isolates from clinical specimens samples in Al-Basrah province, Iraq.
Methods: The current study includes an identification of 100 isolates of coagulase-negative staphylococci (CoNS) that were collected from a variety of clinical specimens such as blood, urine, skin infections, surgical wounds, tracheal and eye swabs). Conventional biochemical tests and the Vitek®2 system were used to identify the isolates to confirm it they're coagulase-negative staphylococci (CoNS). PCR and special primers were used to detect the β-lactamase gene (*blaZ*) and methicillin resistance gene (*mecA*).
Results and Discussion: *S. epidermidis* isolates demonstrated the highest resistance to penicillin 87.2% and the highest sensitivity to cephalexin 64.1%. According to agar screening, 51.3% of *S. epidermidis* isolates gave positive results for methicillin-resistant. From the 39 *S. epidermidis* isolates examined by PCR, the 82.1% were gave positive results for *blaZ* gene and 59% gave positive results for the *mecA* gene.
Conclusions: Prevalence s of mecA gene and blaZ gene between the *S. epidermidis* isolates gave the alert to increase the virulence of *S. epidermidis*, also the used of PCR technique gave the more accurate results for detect mecA gene and blaZ gene in clinicals isolates and prevents the therapeutic failure in hospitals.

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Introduction

For a long time, it was considered that coagulase-negative staphylococci (CoNS), particularly *Staphylococcus epidermidis*, were a crucial part of the normal epithelial flora present in all humans in different parts of the body, including the nares, head, and axilla, and that their presence was essential to the maintenance of healthy skin. *S. epidermidis* is now recognized as one of the most common causes of nosocomial and implant-associated infections [1, 2]. *S. epidermidis*, is a common cause of nosocomial infections and blood infections. It has been isolated from various infections including wounds, skin infections, endocarditis, bacteremia, pneumonia, urinary tract infections, and soft tissue [3].

Penicillin was once the most common therapy for treating *Staphylococcus* infections; however, since 1968, penicillin resistance has increased significantly in CoNS [2, 4]. Penicillin resistance in staphylococci is conferred by two processes. The first and most significant mechanism concerns the development of β-lactamase, which hydrolyses the β-lactam ring of penicillin causing it ineffective [5, 6]. The second is mainly associated with human isolates and presents resistance considering *mecA* encodes PBP2a, a penicillin-binding protein [7]. Coagulase-negative staphylococci (CoNS) have also been shown to be resistant to penicillin due to *blaZ*, indicating that *blaZ* is one of the primary mechanisms behind penicillin resistance in staphylococci [7]. *Staphylococcus* methicillin resistance is produced by PBP2a expression, which is encoded by the *mecA* gene [8]. The poor affinity of PBP2a, a penicillin-binding protein, for methicillin and other β-lactam antibiotics is related to staphylococci's resistance to them, whereas susceptible staphylococci do not have this protein [9].

There have been reports of resistance gene transfer between CoNS and *S. aureus*, indicating that CoNS could serve as a reservoir for the spread of these genes. This means that, given a chance bacterial condition of use, staphylococci of various species may exchange *mecA* and *blaZ* in the same habitat [6, 10]. CoNS is an important reservoir for mobile genetic elements that give resistance to tetracyclines, aminoglycosides, quinolones, β-lactams, and macrolides [10]. These microorganisms use a variety of antibiotic resistance strategies to make themselves resistant to numerous antibiotics. These strategies include the synthesis of enzymes that deactivate drugs, modifications to antibiotic targets, and a decrease in intracellular antibiotic concentration [11, 12].

Therefore, the aim of current study determine resistance to methicillin and antibiotic susceptibility pattern of β-lactam drugs and detect *mecA* and *blaZ* among *S.epidermidis* isolates from clinical specimens samples in Al-Basrah province, Iraq.

Materials and Methods

Bacterial Isolates

From October 2023 to January 2024, a total of 100 coagulase-negative staphylococci (CoNS) isolates have been collected from a variety of clinical specimens (including skin infections, eye, surgical wounds, tracheal, blood and urine), in the Al-Basrah province Iraq. The *S. epidermidis* isolates were identified by the conventional bacteriological methods, including grown on mannitol salt agar, catalase test, tube coagulase and urease tests according to [13]. The Vitek®2 system (Vitek®2 GP ID-P Reference number 21342, bioMérieux, USA), completed the second steps, which included the confirmed identity. The isolates were kept in brain heart infusion (BHI) medium with 15% glycerol at -20 °C.

Antibiotic susceptibility pattern of β-lactam

Kirby-Bauer disc diffusion technique was used to determine the antibiotic susceptibility pattern of β-lactam antibiotic. The following antibiotics were tested: Penicillin (10 µg), Ceftriaxon (30µg), Amoxycillin (10µg), Cefoxitin (30µg),Cefotaxime (30µg), Cephalexin (30µg), and Cephazolin (30 µg) [3].

Detection of methicillin-resistant

Cefoxitin diffusion disc method

The test was performed for *S. epidermidis* isolates using Mueller-Hinton agar plates (LAB - media, England) and antibiotic disks cefoxitin (30 µg) were used to detect the sensitivity pattern according to CLSI- guidelines [14, 15].

Oxacillin agar screen method

The test was performed for *S. epidermidis* isolates using an oxacillin agar screen plate (Mueller-Hinton agar supplemented with 4% NaCl and 6 µg of oxacillin per ml) and by microbroth dilution were performed in accordance according to (NCCLS) guidelines [16].

DNA extraction

Genomic DNA extracted from isolates according to (Wizard® Genomic DNA Purification Kit,Promega, USA) kit protocol.

mecA and *blaZ* genes Detection

The polymerase chain reaction technique has been used to identify the methicillin-resistant *mecA* gene and β-lactamase resistant *blaZ* gene in *S. epidermidis* isolates according to the method of [17, 18] respectively. The primers utilized for amplification of *mecA* and *blaZ* genes were mentioned in the (Table 1).

Table 1: Specific primers of the *mecA and blaZ* genes used in PCR

Primers	Sequence	length	Size (bp)	Optimizing Ta*
mecA-F	5'-AAAATCGATGGTAAAGGTTGGC-3'	22	533	53 °C
mecA-R	5'-AGTTCTGGAGTACCGGATTTGC-3'	22		53 °C
blaZ	5'- CAAAGATGATATAGTTGCTTATTCTCC -3'	27	421	50 °C
blaZ	5'- TGCTTGACCACTTTTATCAGC -3'	21		50 °C

* Ta: Annealing temperature

Results

The 86 isolates of coagulase-negative staphylococci (CoNS) isolates were collected between October 2023 and January 2024. The CoNS isolates distributed to 32(37.2%) urine, 20 (23.3%) blood, 14(16.3%) skin infections, 12(14%) surgical wounds, 5(5.8%) tracheal and 3(3.4%) eyes. Identification of bacterial growth by using biochemical tests and Vitek®2 have been revealed as follow *Staphylococcus epidermidis*: 39 (45.3%), *Staphylococcus haemolyticus*: 28 (32.5%), and *Staphylococcus saprophyticus*: 11(12.8%), were the most frequent bacterial species followed by *Staphylococcus warneri:* 4 (4.7%), *Staphylococcus schleifericus:* 3 (3.5%), and *Staphylococcus capitis:* 1(1.2%) (Figure 1).

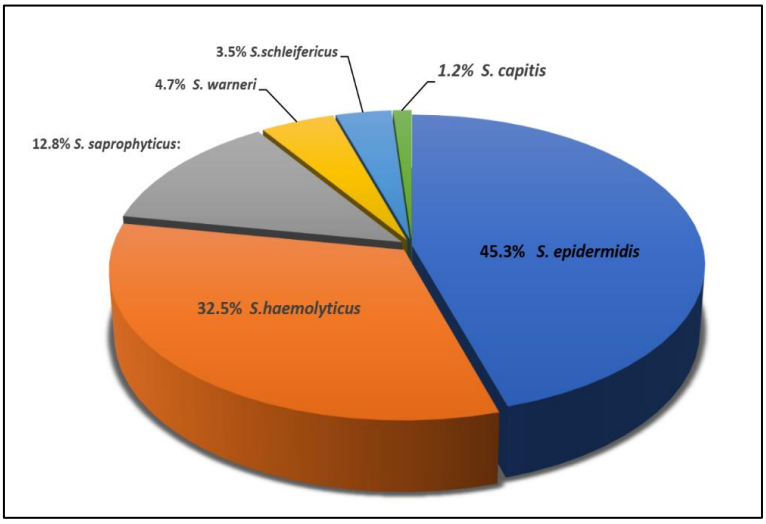

Fig 1: The frequency of coagulase-negative staphylococci (CoNS) isolates

The distributed of 39 *S. epidermidis* isolates in different clinical specimens, in current study were shown the majority of specimens have been identified in urine 15(38.5%) and blood 9(23.1%), follow by skin infections 7(17.9%) and surgical wounds 5(12.8%), while the lowest percentage of specimens were found in tracheal 2(5.1%) and eye samples 1(2.6%) as in (Figure 2).

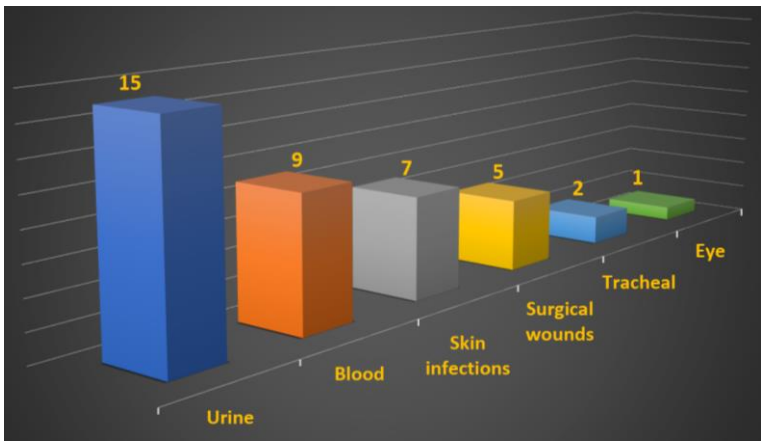

Fig 2: Distribution of *Staphylococcus epidermidis* isolated from clinical samples

Furthermore, Kirby-Bauer disc diffusion technique was used to determine the antibiotic susceptibility pattern of β-lactam antibiotic, the *S. epidermidis* isolates showed higher resistance results to penicillin 34(87.2%) compared to ceftriaxone 31(79.5%), and higher sensitivity to cephalexin 25(64.1%) compared to Cephazolin 22(56.4%) (Table 2).

Table 2: β-lactam antibiotics resistance pattern *Staphylococcus epidermidis* isolates

No.	Antibiotic	*Staphylococcus epidermidis n=39*		
		Resistant	**Intermediate**	**Sensitive**
1	Penicillin	34(87.2%)	0	5(12.8%)
2	Ceftriaxone	31(79.5%)	2(5.1%)	6(15.4%)
3	Amoxycillin	27(69%)	0	12(31%)
4	Cefoxitin	24(61.5)	4(10.3%)	11(28.2%)
5	Cefotaxime	21(53.8%)	1(2.6%)	17(43.6%)
6	Cephalexin	11(28.2%)	3(7.7%)	25(64.1%)
7	Cephazolin	16(41%)	1(2.6%)	22(56.4%)

Furthermore, out of n=39 *S. epidermidis* isolates tested for detection of methicillin-resistant by using cefoxitin diffusion disc method, the 20 (51.3%) *S. epidermidis* isolates showed positive results for methicillin-resistant, while 19 (48.7%) *S. epidermidis* isolates gave negative results. Whereas, using oxacillin agar screen method was gave 17 (43.6%) *S. epidermidis* isolates showed positive results for methicillin-resistant, and 22 (56.4%) *S. epidermidis* isolates gave negative results (Table 3).

PCR results of amplified *blaZ* gene were showed the 32(82.1%) *S. epidermidis* isolates had gave positive results for *blaZ* gene, while the 7(17.9%) *S. epidermidis* isolates had gave negative results for *blaZ* gene. Furthermore, the results of amplified *mecA* gene were showed the 23 (59%) *S. epidermidis* isolates had gave positive results for the *mecA* gene, while the 16(41%) *S. epidermidis* isolates had gave

negative results for *mecA* gene.

Table 3: Methicillin resistance pattern for *Staphylococcus epidermidis* isolates using conventional methods and PCR Technique

Parameter	Methods used for detection of MRSA		
	Cefoxitin diffusion disc method	Oxacillin agar screen method	PCR Technique
Methicillin resistance *S. epidermidis*	20 (51.3%)	17 (43.6%)	23 (59%)
Methicillin sensitive *S. epidermidis*	19 (48.7%)	22 (56.4%)	16(41%)

Discussion

Recently, the role of coagulase-negative staphylococci; CoNS, as opportunist microbial flora, has become much more prominent in nosocomial infections. Due to nosocomial infections are largely caused by coagulase-negative staphylococci (CoNS), accurate detection of bacterial strains in laboratories is essential. Due antibiotic resistance is increasing and infections are becoming more resistant to medication, that needs more develop improved diagnosis and more efficient antimicrobial therapy, particularly in hospital settings [6]. The CONS strains are often reported nosocomial infections; they are common mucous and skin membrane pathogens. Methicillin resistance in particular poses a difficulty in the therapeutic management of these infections [5].

The results in current study were showed among of 39 *S. epidermidis* isolates the highest percentage in urine (38.5%) and blood samples (23.1%). The [3, 19, 20] studies were reported the highest isolation in urine and blood samples. Together, these three studies results align with current study observations. It is important to pay attention to the increasing rate of β-lactam resistance in *S. epidermidis* isolates. In the present study, depending on phenotype tests the results showed the 87.2% and 79.5% of *S. epidermidis* isolates were gave resistance to Penicillin and Ceftriaxone. On other hand the results were showed among the 39 *S. epidermidis* isolates in current study, 82.1% contained *blaZ*, which is agreement with reported [3, 21, 22] a varying range of resistance to β-lactams in *S. epidermidis* isolates. Also [20] reported the highest resistance to Penicillin and Ceftriaxone, while the least resistance to Ciprofloxacin and Cephalexin. Furthermore, [22] reported the highest resistance to Penicillin, Methicillin, Ceftriaxone and Ceftizoxime, and the lowest to Cephazolin, Cephalexin respectively.

MRSA has become one of the most frequent nosocomial infections. It is critical to diagnose MRSA early and implement an antimicrobial therapy strategy [23, 24]. *S. epidermidis* resistance to methicillin has increased globally [25], possibly due to the transfer of a *mecA* gene from the species to *S. aureus* through horizontal gene transmission [26]. In this study, approximately 70 % of *S. epidermidis* isolates carried the *mecA* gene. The [27-29] reported the harbored of *mecA* gene among the *S. epidermidis* in different percentage (64.0%, 75.43% and 70.7%,), respectively. Different studies indicated a *S. epidermidis* had high percentages of resistance against methicillin and carried *mecA* gene, [30] was found the *mecA* gene in 95.8% of *S. epidermidis* isolates; also [31] the *S. epidermidis* giving (93.75%) positive results of *mecA* gene. The studied of [32, 33] reported that (85%, 92.2%) of isolates harbored *mecA* gene respectively. Other studies [34, 35] reported a low of *mecA* gene existence, the (34.4%,10%) of isolates harbored *mecA* gene respectively.

The results of PCR analysis in current study confirmed the presence of *blaZ* gene in 32(82.1%) *S. epidermidis* isolates, while the 7(17.9%) *S. epidermidis* isolates had gave negative results for *blaZ* gene, and these results were similar with study of [3]. Study of [36] found that 149 specimens out of 198 specimens (75.25%) contained *blaZ* gene. Resistance among isolates to different β-lactam antibiotics is increasing. As a result, an accurate and accurate estimation is necessary for the treatment of illnesses linked to *S. epidermidis* infections [3, 7, 20]. Furthermore, the results of amplified *mecA* gene were showed the 23 (59%) *S. epidermidis* isolates had gave positive results for the *mecA* gene, while the 16(41%) *S. epidermidis* isolates had gave negative results for *mecA* gene. Study of [12] was reported Out of 28 CoNS isolates, 15(53.57%) were methicillin resistant coagulase-negative staphylococci (MRCoNS) isolates and 13(46.43%) were methicillin sensitive coagulase-negative staphylococci (MSCoNS) isolates. The [37] study was found MRSA in 55.7 % and 48.7% of inpatients and outpatients, respectively. The [38] found a prevalence of 54.9%, and also 62.4% in MRSA reported from Iran [39]. While [40] in north India methicillin resistant was 38.8%.

Conclusions

Prevalence s of *mecA* gene and *blaZ* gene between the *S. epidermidis* isolates gave the alert to increase the virulence of *S. epidermidis*, also the used of PCR technique gave the more accurate results for detect *mecA* gene and *blaZ* gene in clinicals isolates and prevents the therapeutic failure in hospitals.

Ethical approval

This study was approved by the ethics committee of college of science, department of Pathological analyses**,** university of Al-Basrah.

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