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Molecular Surveillance of *mecA*, *mecC*, and *blaZ* Genes in Urpathogenic Coagulase-negative *Staphylococci* Isolated from Woman Outpatients in Wasit Province, Iraq

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Abstract

Urinary tract infections caused by coagulase-negative staphylococci have increased, accompanied by raising antibiotic resistance particularly during pregnancy. This study aimed to investigate the prevalence of *mecA*, *mecC*, *blaZ*, and *vanA* genes in *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from young female outpatients with acute UTI. Hence, 26 *S. epidermidis* and *22 S. haemolyticus* isolates were surveyed in this study. *mecA* gene was identified in both *S. epidermidis* 88.4% and *S. haemolyticus* 86.3% isolates, with no significant difference between the two species. The *blaZ* gene was present in 96.3% and 81.8% of *S. epidermidis* and *S. haemolyticus* isolates, also the difference was not significant. All *mecA*-positive *S. epidermidis* isolates and 84.2% of *mecA*-positive *S. haemolyticus* isolates co-carried *blaZ* gene. The *vanA* and *mecC* genes were absent from all isolates. These findings highlight the emergence of *β*-lactams' resistance among CoNS isolated from young women outpatients with acute UTI in the study area.

Keywords: Coagulase-negative *Staphylococci*, UTI, *mecA*, and *blaZ*.

1. Introduction

Worldwide, urinary tract infection (UTI) affects approximately 150 million patients each year with a recurrence rate of 27% among women within six months of the first episode [1]. Among sexually active women, *Staphylococcus spp*. represents the second most common cause of uncomplicated UTIs. The most frequently isolated species are *S. saprophyticus*, *S. aureus*, *S. warneri*, *S. epidermidis*, *S. hominis*, *S. lentus*, and *S. haemo-lyticus* [2].

The capability of *Staphylococci* to generate resistance to antibiotics especially beta-lactams antibiotics is through the expression of *blaZ* gene that encodes

resistance to penicillin by the production of beta-lactamases and the *mecA* gene that encodes for methicillin resistant through the production of PBP2a [3]. The *mecA* gene which encodes for a penicillin binding protein (PBP2') is a part of a mobile genetic element known as *Staphylococcal* cassette chromosome (SCCmec). In addition to *mecA*, in 2011, reports of new MRSA strains carrying a *mecA* variant, was highly significant.

The novel *mecC* gene showed approximately 69% DNA identity with the classic *mecA* gene [4]. The methicillin and vancomycin resistance are increasingly reported. Vancomycin resistance is mediated by the *vanA* gene and lesser extent by *vanB* [5]. Despite the global significance of this problem, there is a lack of data from Iraq, particularly regarding the molecular mechanisms of resistance in uropathogenic CoNS. Therefore, this study was designed to investigate the co-occurrence of *mecA*, *mecC*, *blaZ*, and *vanA* genes in S. *epidermidis* and *S. haemolyticus* isolated from female outpatients with acute UTI.

2. Materials and methods

2.1 Bacterial isolates

A total of 48 *staphylococcal* isolates, comprising *S. epidermidis* (26 isolates) and *S. haemolyticus* (22 isolates), were enrolled in this study. The isolates were obtained from the Microbiology Laboratory, Department of Biology, College of Science, Wasit University. Samples isolated from young women (18-40 years old) outpatients with symptomatic acute UTI between July 2023 to January 2024. Description of patients' information and isolation and identification of the isolates were published previously [6].

2.2 DNA Extraction

Total DNA was extracted using a boiling method adapted from [7]. Briefly, three loopfuls of an overnight culture grown on tryptic soy agar were suspended in 1ml of sterile 1X TE buffer (pH 8). The suspension was boiled in a water bath at 85˚C for 20 minutes and then immediately transferred to an ice bath for 10 minutes. After centrifugation at 10,000 rpm for 5 minutes. The supernatant was aliquoted 200µl and stored at - 20˚C till use.

2.3 Detection of *mecA*, *mecC*, *blaZ* and *vanA* Genes

Conventional PCR was used to detect *mecA* and *mecC* genes according to [4] and the *blaZ* and *vanA* genes according to [3]. The specific primers and PCR amplification conditions are listed in tables 1 and 2. Following amplification, 10 µL from each reaction was subjected to electrophoretic separation on a 1.5% or 2%

agarose gel stained with ethidium bromide and visualised under ultraviolet light.

Table 1: Target genes and their primers.

Gene	Primer	Primer sequence (5′- 3′)	Product size bp
mecA	F	ACGAGTAGATGCTCAATATAA	293
	R	CTTAGTTCTTTAGCGATT	
mecC	F	GCTCCTAATGCTAATGCA	584
	R	GGCTTAGAACGCCTCTATGA	
blaZ	F	GTTGCGAACTCTTGAATAGC	674
	R	GGAGAATAAGCAACTATATATCATC	
vanA	F	GGGAAAACGACAATTGC	732
	R	GTACAATGCGGCCGTTA	

Table 2: PCR Conditions.

PCR conditions	*mecA*/*mecC*	*blaZ*	*vanA*
Initial denaturation	94 °C/5 min	94 °C/5 min	94 °C/2 min
Denaturation	94 °C/30sec	94 °C/1min	94 °C/1 min
Annealing	60 °C/30sec	54 °C/1min	54 °C/1 min
Extension	72 °C/45sec	72 °C/1min	72 °C/1 min
Number of cycles	30	35	30
Final Extension	72 °C/5min	72°C/10min	72 °C/10min

2.4 Statistical Analysis

The Chi square test (SPSS software, version 2.1, IBM, NC, USA) was applied to determine the statistical significance of difference in the prevalence of the studied criteria with a P value of ≤ 0.05 considered significant.

3. Results and Discussion

3.1 Prevalence of mecA gene

In the present study, the results demonstrated the prevalence of *mecA* gene (figure 1) in 88.4% (23/26) of *S. epidermidis* isolates and in 86.3% (19/22) of *S. haemolyticus* isolates, no statistically significant difference between species. In contrast, the *mecC* gene was totally absent from all isolate.

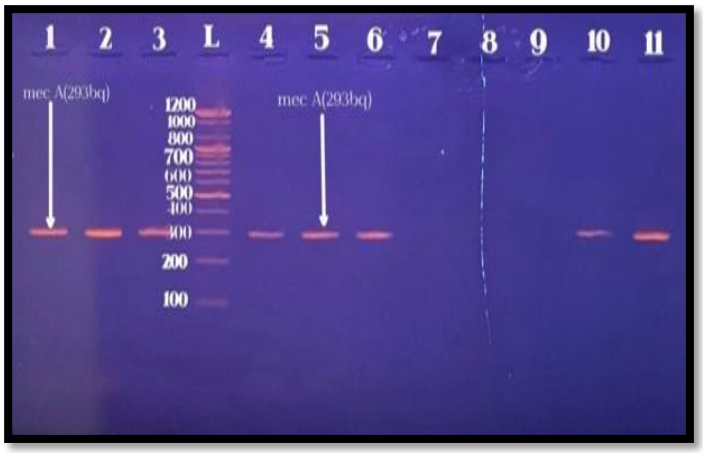

Figure 1: Electrophoresis of *mecA* gene amplification shows *mecA* band at 293 bp.

Results of the current study revealed a dominance of *mecA* gene among uropathogenic staphylococcal isolates. in *S. epidermidis*, 88.4% of the isolates were positive for *mecA* gene, this prevalence is slightly higher than the 80% reported in Iraq [8]. In contrast, a study in Tanzania [1] found that only 4.3% of isolates harboured the gene. For *S. haemolyticus*, 86% of the isolates were positive for the *mecA* gene.

In Iraq [9] 100% of *S. haemolyticus* bloodstream isolates harboured *mecA* gene. In Saudi Arabia [10] *mecA* gene was found in 100% of *S. haemolyticus* isolates, while this gene was reported in 77.7% of this species isolates [1]. This variation in prevalence could be explained by

geographic differences, the state of host immunity, environmental exposure, and the level of bacterial virulence [11]. In the current study, the *mecC* gene was totally absent from all the isolates.

This finding aligns with a study from Iraq [12] and Turkey [13], where they reported the absence of *mecC* from methicillin-resistant staphylococci (MRS) from animal and human samples. The absence of *mecC* in this study indicated its rare presence in human clinical staphylococcal isolates, particularly in urban areas where human-livestock is limited. These observations support the hypothesis that the distribution of antibiotic resistance genes varies geographically and is strongly influenced by ecological and socio-cultural factors, particularly the extent of human-livestock contact [14].

3.2 Prevalence of *blaZ* gene

Regarding *blaZ* gene, the present study confirmed the prevalence of this gene in 96.3% (25/26) and 81.8% (18/22) of *S. epidermidis*, and *S. haemolyticus* isolates, respectively, with no significant difference between the two species (figure 2).

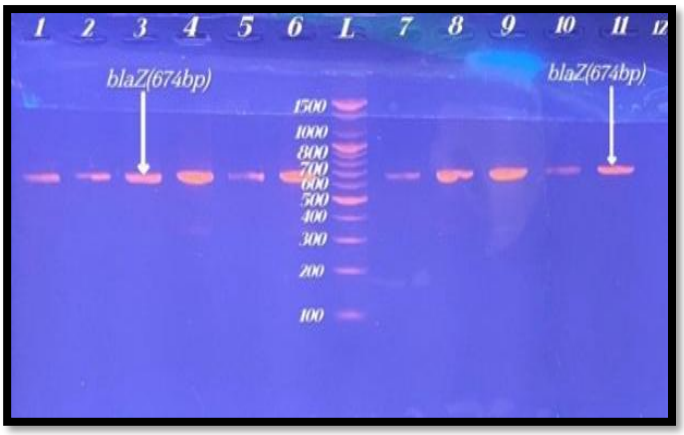

Figure 2: Electrophoresis of *blaz* gene amplification shows *blaZ* band at 674bp.

The *blaZ* gene was found in 96.3% of *S. epidermidis* isolates. This result aligns closely with a study from Iraq [15] which reported a 91.7% prevalence in isolates from wound infections. A similar rate 80% was reported in Iran [16] for isolates from various clinical specimens. For *S. haemolyticus*, 81.8% of isolates in this study were positive for the *blaZ* gene. This prevalence is higher than the 57.5% reported in Iraq [17] for isolates from vaginitis cases.

Also in Tanzania [1], 77.8% of S. *haemolyticus* isolated were positive for *blaZ* gene. Overall, the findings from most regions are consistent with the high prevalence of the *blaZ* gene observed in the present study. This high prevalence indicates that *S. epidermidis* and *S. haemolyticus* can hydrolyse the amide bond in *β*-lactam ring through the production of *β*-lactamase enzyme [18].

3.3 Prevalence of *vanA* gene

In the current work, the *vanA* gene was absent from all staphylococcal isolates. The absence of *vanA* gene in the present study is consistent with the findings in Saudi Arabia [10] and Pakistan [19], where there was no vancomycin resistance in uropathogenic *S. haemolyticus* and *S. epidermidis*. The absence may be due to the restricted use of vancomycin for treating UTI in pregnant women, owning to the drug's potential adverse effects on fatal health [20].

3.4 Co-occurrence of *mecA* and *blaZ* genes

The co-occurrence of *mecA* and *blaZ* genes was observed in 100% of *S. epidermidis* and 84.2% of *S. haemolyticus* isolates. The *blaZ* gene was harboured by 100% and 84.2% of *mecA*-positive *S. epidermidis* and *S. haemolyticus* isolates, respectively. This co-occurrence of these genes suggests a dual resistance mechanism, which further reduces the clinical efficacy of *β*-lactam antibiotics and renders them ineffective against infections caused by these isolates.

Similar findings have been documented in other regions. For instance, In Iraq [21] and in Nigeria [22]. The consistency between these reports and the present study underscores the global distribution of co-existing staphylococcal resistance mechanisms and highlights the growing therapeutic challenge they pose.

4. Conclusion

This study demonstrated a high prevalence and co-occurrence of both *mecA* and *blaZ* genes among uropathogenic *S. epidermidis* and *S. haemolyticus* isolates from young women in Wasit Province, Iraq. However, these findings suggest a need for continuous molecular surveillance, rational antibiotic use, and strengthened infection control strategies to prevent the further dissemination of resistant strains.

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