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Detection of some Staphylococcal Cassette Chromosome *mec* (SCC *mec*) Genes in *Staphylococcus epidermidis* and its relationship to Antibiotic Resistance

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Abstract

Background: *Staphylococcus epidermidis* is consider a significant microbiome agent have sudden emerging roles [1]. And CoNS were considered a reservoir of antimicrobial resistance. The Methicillin Resistance controlled by the *mecA* gene, which encodes a low-affinity protein to *β*-lactam antibiotics, called penicillin-binding protein (PBP2a). *mecA* gene locate on the Staphylococcal Cassette Chromosome *mec* (SCCmec) element, a genomic island distributed widely among staphylococci [2].

A mobile genetic element called SCC was mediate the methicillin resistance in staphylococcus species strains. SCC*mec* consists of two genetic elements: the first expresses the methicillin resistance that is called *mec*, and the second that expresses the integration and excision responsibility of SCC*mec* in a bacterial genome, that called ccr complex[8][9].

Objective: Evaluate the contribution of some SCC*mec* genes (*mec*I, *ccr*A, *ccr*B, *cch*) in antibiotic resistance of different clinical infections caused by *Staphylococcus epidermidis*.

Methods and Results: A three hundred clinical samples collected then cultured on different media and examined by gram stain, catalase test, and bound coagulase test for diagnosed the *Staphylococcus epidermidis* presence as infectious or opportunistic agent. Then verified the diagnosis by Analytical Profile Index system that certain only 3 isolates were *Staphylococcus epidermidis* from total isolates. Then, bacterial genome extracted by Genomic DNA Extraction Kit (G-spin™/ Pioneer/ Korea). Then, conventional PCR was carried out to total genes and the results interpreted by agarose gel electrophoresis (Labnet/ United State America) and pictured by UV transilluminator (Photo Gel Detector system/ Elettrofor/ Italy). The final results showed these genes (*ccr*A, *ccr*B, *cch*) involved by 33.3% of absolutely resistance processing of Azithromycin, Cefotaxime, and Cefixime.

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Key words: *Staphylococcus epidermidis*, SCCmec genes (*mec*I, *ccr*A, *ccr*B, *cch*), Analytical Profile Index Staph system.

Introduction: Staphylococcus epidermidis consider an important member of coagulase-negative staphylococci, is a significant microbiome agent of human skin and mucous membrane; and sudden emerging evidence of its roles for human health in fighting off harmful agents [1].

As CoNS retain several antimicrobial genes, it's considered a reservoir of antimicrobial resistance. One of the most complicated public health matter is the methicillin resistance (MR). The MR feature is controlled by the *mecA* gene, which encodes a low-affinity protein to β-lactam antibiotics, called penicillin-binding protein (PBP2a). *mecA* gene holds on the staphylococcal cassette chromosome mec (SCCmec) element, a genomic island distributed widely among staphylococci [2].

SCC*mec* has been defined in six different allotypes in *Staphylococcus aureus* and five different allotypes in CoNS, depending on the combination of the *mec* gene complex class and the cassette chromosome recombinase (*ccr*) gene complex type [3].

The regulation of *mec*A gene expression and the PBPs production is proceeded by proteins encoded by the inducer-repressor system of penicillinase-associated *bla*R1-*bla*I, and the resembling genomic *mec*R1-*mec*I elements. The repressor protein encodes by *mec*I gene and a *β*-lactam-sensing transmembrane signalling protein encodes by *mec*R1 gene. Consideration of methicillin and oxacillin as weak inducers for this system, because resulting in sluggish induction of methicillin resistance. Discovering of strains that have phenotype sensitivity, famous as pre-methicillin-resistant *S. aureus* (pre-MRSA) and pre-methicillin-resistant coagulase-negative staphylococci (pre-MRCoNS), which do not exhibit methicillin resistance, due to fully repression of *mec*A by *mec*I. The induction was very slow of *mec*A transcription, might be caused mutations in *mec*I [10].

Materials and Methods:

- **Samples Collection:** A three hundred samples were collected from different clinical sites of the patients who attended to one hospital in Al-Najaf Province, at the period from the first of September (2020) to the final of December (2020). All three hundred patients surely not administered any antibiotics before at least one week to the culture date.
- **Bacterial isolation and Identification:** By different culture media and biochemical test as catalase test, bound coagulase test (slide method) and also by gram staining, the bacterial isolates were primary isolated. The

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identification was done by Analytical Profile Index system depending on the procedure of manufacture (BioMérieux/ France).

• **Antibiotic Susceptibility:** By Kirby-Bauer method (a single disc diffusion test) the antibiotic sensitivity was done to antimicrobial agents. And depending on Clinical Laboratory & Standard Institute (CLSI 2019) measuring the inhibition zones around the antibiotic disc was carried out.

• **Molecular Detection:**

- ❖ **DNA Extraction:** Genomic DNA Extraction Kit (G-spin™/ Pioneer/ Korea) was used to extract genomic DNA from bacterial isolates.
- ❖ **Amplification Protocol:** A forward and reverse primers were used as in table (1), to amplified the target genes.

Table (1) primers used in this study.

Name of gene	5′ -- 3′ Sequence	Amplicon size	Reference
*mec*I	F:GGTTATGTTGAAACGAAAG R:GGTGTTATTACAAGCATTATTG	637 bp	[4]
cch	F:MAATCGTGAASAWGAAGTYATTMAATGGTT R:GCAATSATTTTBACYTSGAT ATGRTYATCTT	371 bp	[5]
*ccr*A	F:TCRGADAAYCARCTVAAACAAAARATCAAATG R:TATAGGGRTRCARYATGTTTARCGTGAAAC	477 bp	
*ccr*B	F:TATCGTAAAATAGCSAATGCAYTVAATCACAAAGG R:ACTTTATCACTTTTGACAATTTCRAGTATTTG	513 bp	

A conventional Polymerase Chain Reaction (Techne/ United State of America) was carried out to amplification of target genes by PCR Thermocycler Programs as in below table.

Table (2) Polymerase Chain Reaction Thermocycler Programs

PCR step	Temperature/ Time	Repeat
Initial Denaturation	95 ◦C/ 5min for all genes	1
Denaturation	94 ◦C/ 30S for all genes	35 Cycles
Annealing	55 ◦C/ 30S for *ccr*A, *ccr*B, *cch*	
	51.6 ◦C/ 40S for *mec*I	
Extension	72 ◦C/ 45S for *ccr*A, *ccr*B, *cch*	
	72 ◦C/ 60S for *mec*I	
Final Extension	72 ◦C/ 10min for *ccr*A, *ccr*B, *cch*	1
	72 ◦C/ 7min for *mec*I	

****** The *mec*I Thermocycler Program [4].
*The *ccr*A, *ccr*B, *cch* Thermocycler Programs [5].

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- **Electrophoresis:** By Agarose gel electrophoresis (Labnet/ United State America) the PCR products were analysed. And the images was taken to the results by UV transilluminator (Photo Gel Detector system/ Elettrofor/ Italy).

• **Ethical Approval:** The consent of all patients included in the research study was taken.

• **Results and Discussion:** This study was done during the period from the first of September (2020) to the final of December (2020). A three hundred patients sampled from different clinical sites. Results of present study showed the ratio of infection in females (199, 66%) higher than in males (101, 34%) as shown below.

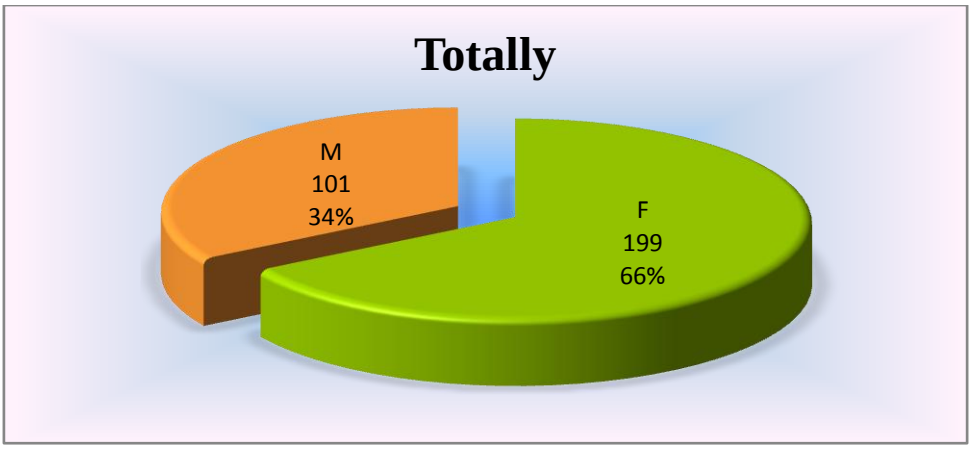

Figure (1): Distribution of infection according to Patients Gender.

The bacterial infections distribution depend on sexual dimorphism and has been greatly attributed to sex hormones levels differences between males and females, as well as to genetic factors. In common, males are more sensitive to gastrointestinal and respiratory bacterial infection and sepsis, whereas females are more sensitive to genitourinary tract infection[6].

Antimicrobial Susceptibility: Most isolated bacterial strains were resistant to Cefixime (93.1%), while most isolated bacterial strain were susceptible to Nitrofurantoin (63.9%). Whereas another antibiotics showed different resistance ratios in isolated bacterial strains, there were (17.3%, 21.4%, 22.7%, 45.9%, 48.9%, 66.3%, 67.8%, 69.2%, 76.7%), Gentamicin, Ciprofloxacin, Nitrofurantoin, Trimethoprim, Norfloxacin, Azithromycin, Ceftriaxone, Cefotaxime, Amoxicillin, respectively.

The factors leaded to antibiotic resistance was insufficient directive, usage inaccuracies, and consciousness lack in best practices which guidance unnecessary or wrong use of antibiotics [7].

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SCC *mec* genes (*mec*I, *ccr*A, *ccr*B, *cch*): In current study the *Staphylococcus Cassette Chromosomes mec* genes (*mec*I, *ccr*A, *ccr*B, *cch*) was investigated by conventional PCR method to study antibiotic resistance relationship of interested bacterial species that is *Staphylococcus epidermidis*, which resulted the identification of only three isolates of 300 patients of totally study samples as *Staphylococcus epidermidis*.

Table (2): The *Staphylococcus epidermidis* isolates and antibiotics survey and detected genes.

Bacterial Isolates	Amoxicillin	Azithromycin	Ciprofloxacin	Ceftriaxone	Cefotaxime	Cefixime	Gentamicin	Nitrofurantoin	Norfloxacin	Tri-methoprim	*mec*I	*ccr*A	*ccr*B	*cch*
S. epidermidis	R	R	R	R	R	R	R	R	R	M	-	-	-	-
S. epidermidis	R	R	M	S	R	R	S	R	R	S	-	+	+	+
S. epidermidis	M	R	S	M	R	R	S	S	S	S	-	-	-	-

All *Staphylococcus epidermidis* isolates were completely resistant to Azithromycin, Cefotaxime, and Cefixime (100%), and only one isolate showed resistance to these antibiotics and retained resistance genes (*ccr*A, *ccr*B, *cch*) meaning that 33.3% of resistance to Azithromycin, Cefotaxime, and Cefixime (resistance absolute, 100%) was due to the presence of *ccr*A, *ccr*B, *cch* genes in these bacterial strains.

Despite the lowest detection ratio of (*ccr*A, *ccr*B, *cch*) genes but it's still a source of antibiotic resistance, and it may transferred horizontally or vertically to other bacterial strains.

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