*Journal of Advances in Medicine and Medical Research*

***24(10): 1-11, 2017; Article no.JAMMR.38128***
***ISSN: 2456-8899***
*(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)*

# Variety of the Structures of Staphylococcus Cassette Chromosome Mec in Coagulase- negative Staphylococci and Their Effects on Drug Resistance

**Alia Essa Shams Aldeen[1*] and Kareem Thamer Meshkoor[1]**

*[1]Department of Microbiology, College of Medicine, University of Kufa, Iraq.*

***Authors' contributions***

*This work was carried out in collaboration between authors. Author KTM designed the study wrote the protocol and wrote the first draft of the manuscript. Author AESA managed the analyses of the study. Managed the literature searches. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JAMMR/2017/38128
Editor(s):
(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.
Reviewers:
(1) J. A. A. S.Jayaweera, Rajarata University of Sri Lanka, Sri Lanka.
(2) K. Muddukrishnaiah, Kerala University of Health science, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/22391

***Original Research Article***

***Received 13th November 2017***
***Accepted 13th December 2017***
***Published 20th December 2017***

## ABSTRACT

**Background:** In the past coagulase negative staphylococcus were often seen as contaminants, but were later considered one of the most common photogenic bacteria in the hospital over the last decade Identification of beta-lactam and particularly methicillin in hospitals is of concern. The fundamental principle for the treatment of CoNS is the rapid identification of resistance mechanisms particularly their resistance to methicillin. The objective of this study is to determine the antibiotic resistance, the frequency of the *mecA* gene, and to determine the types of *SCCmec* in the CoNS isolates of from clinical isolates and control.
**Study Design:** Point prevalence case-control study.
**Place and Duration of Study:** Breast milk samples were collected from (200) patients suffering from mastitis and 106 lactational women as control whom visit center of breast examination in hospital Al- Sadder –in Najaf- Iraq, during the period from July/ 2015 to Jun/ 2016.
The standard biochemical tests were used for all isolates are coagulase, catalase, oxidase, and modified oxidase the CoNS isolates, then diagnosed by vitek -2 technique and the antibiotic sensitivity testing was carried out also by vitek-2 system. Then genomic isolate were extracted and mecA gene was detected by PCR technique, the types of *SCCmec* were performed by

**Corresponding author: E-mail: aliashams_1970@yahoo.com;*

RFLP-PCR.
**Results:** Among the 62 breast milk isolates (50 mastitis, 12 control), identification of CoNS at the species level indicated that *Staphylococcus epidermidis* was the most common species, with 40 isolates, followed by *Staphylococcus haemolyticus* (10), *Staphylococcus hominis* (12).All isolates appeared had *mecA* gene , no one harbored *SCCmec* type I, 8 (12.9%) harbored SCC*mec* type II, 12 (19.3%) harbored SCC*mec* type III, 30 (48.3%) harbored *SCCmec* type IV and 8 (12.9%) remained non-typable. *Staphylococcus epidermidis* was the most isolates that harbored *SCCmec* type IV.
**Conclusion:** The results showed that CoNS revealed high percentage of resistance to methicillin, and the type III, *SCCmec* type was the most prevalence type of which encodes the largest number of resistance genes. The study give the information could be used in epidemiological study for preventing of infectious control in hospital and health centers.

*Keywords: Diversity of SCCmec; CoNS; drug resistant; mecA gene.*

## 1. INTRODUCTION

Recent years, coagulase-negative staphylococci (CoNS) have emerged as important causative agents of illness, The resistance of CoNS to methicillin and other β-lactam antibiotics is mediated by a penicillin-binding protein which has reduced affinity for these antibiotics *(PBP2a). This protein is encoded by the mecA gene* [1] inserted in a genomic island called staphylococcal cassette chromosome *mec* (*SCCmec) 2.SCCmec* is a mobile genetic element which is a vehicle for exchanging resistance genes between Staphylococcus strains and is widely distributed among CoNS. species and in *Staphylococcus aureus* [2,3,4,5] *SCCmec* was initially described as 'mec DNA', which was found only in methicillin-resistant *S. aureus* (MRSA) and absent in methicillin-sensitive *S. aureus* (MSSA) [3,2]. This genetic element is composed of different combinations between the *mec* complex, which encodes resistance to methicillin, and the *ccr* complex, which encodes recombinase enzymes responsible for the mobility of the genetic element. The *mec* complex is composed of *IS431*, *mec*A and intact or truncated sequences of *mecI* and *mecR1* regulatory genes. The *ccr* complex can be composed of recombinase genes *ccr*A and *ccr*B or *ccr*C [6,7,8,9,10]. Based on such diversity, six types of SCC*mec* have been described in *S. aureus*, and five types (I–V) have also been described in CoNS. Moreover, community methicillin-resistant *S.epidermidis* (C-MRSE) have been found with *SCCmec* type IV [11,12], and SCC*mec* type V has already been discovered in CoNS particularly in *Staphylococcus haemolyticus* 8.

The structure and distribution of *SCCmec* types in *S. aureus* have been studied frequently [7,11,4,13,2], but data related to CoNS are only comparative and have been obtained in studies the purpose of which was to determine whether there was transmission between species [14,11] In this study, we evaluated the distribution of *SCCmec* types I, II, III and IV in methicillin-resistant CoNS obtained from breast milk of mastitis and healthy .who visit the center of breast examination in Al-Sadder hospital in Najaf –in Iraq.

## 2. MATERIALS AND METHODS

### 2.1 Patients and Samples

Breast milk samples were collected from (200) patients suffering from mastitis and 106 lactational women as control whom visit center of breast examination in hospital Al- Sadder –in Najaf- Iraq, during the period from July/ 2015 to Jun/ 2016). Milk samples(2-3ml) were cultured on baired parker agar for identification and enumeration of staphylococci .Milk samples were collected aseptically according to [14] and transferred to the lab in cold condition , samples cultured on baired parker agar for isolation of staphylococci (the colony number <4 cfu/ml$^{-1}$) [15]. Then CoNS were identified by biochemical tests include coagulase, catalase, modified oxidase according to [16], and by vitiek-2 system to species level as well.

### 2.2 Antibiotic Susceptibility Method by Vitek – 2 System

Antibiotic sensitivity testing was performed for isolates that were typed to species level, the isolates were subjected to antibiotic susceptibility testing by using the vitek-2 system (bioMérieux, France), by AST-GP580 Gram positive susceptibility cards. The isolates that tested for antibiotic susceptibility were only those that were

diagnosed for genus and species, which include *S. epidermidis* (40 strains), *S. heamolyticus* (10 strains), *and S.hominis* (12 strains) (about 50 strains from patients with mastitis and only12 strains from healthy women). Antibiotics used included cefoxitin screen (used to confirm the presence of MRSA and detect low level methicillin resistance), benzyl penicillin, oxacillin, mupirocin, clindamycin, inducible clindamycin resistance, tetracycline, as well as aminoglycosides (gentamicin, tobramycin), quinolones (levofloxacin, moxifloxacin), and glycopeptides (teicoplanin, vancomycin) [17].

### 2.3 Molecular Detection of *mecA*

#### 2.3.1 DNA extraction

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Geneaid).

#### 2.3.2 PCR detection of *mecA* gene

The primer specific for amplification of resistant gene (*mecA*) was: forward primer CTTGGGGTGGTTACAACGT and revers primer ACCACCCAATTTGTCTGCCA The PCR condition used to amplify mecA gene involved the following: each 20 ML of PCR reaction contained (5 µl of DNA template, 10 pmol of forward primer, 10 p mol reveres primer, and 12.5 µl master mix, then volume was completed with molecular grade water. The PCR amplification product expected to be 541 base pair (bp) was visualized by electrophoresis on 2% agarose gel, at 100 volt and 80 AM for 1hr. The size of amplicon was determined in comparison to the 100bp ladder (Promega, USA).

### 2.4 RFLP-PCR Technique Used for Determination of SCC*mec* Type

The *SCCmec* type was RFLP-PCR technique was performed for genotyping of staphylococcal cassette chromosome *mec* (*SCC mec)* based restriction of *ccrB* gene forward-GCTATTATCAAGGCAATTTACC and revers CTTTATCACTTTTGACTATTTCG (643bp) [18] in coagulase negative staphylococcus bacterial isolates. This methods were carried out according to described by [15].

| Restriction enzyme | Country/Company |
|---|---|
| *HinFI* | Biolab/UK |
| *BsmI* | Biolab/UK |

## 3. RESULTS

### 3.1 Distribution of Coagulase Negative Staphylococci

A total of 88 isolates of CoNS isolated in pure culture, from breast milk of lactating women (66 patients with mastitis and 22 healthy as control) included in the study as shown in Table 1.

The distribution of CoNS as follows: *S. epidermdis* 40 strains, followed by *S. hominis* 12 strains, *S. haemolyticu*s 10 strains, and Other coagulase negative staphylococci 24 strains recovered for patients suffering from mastitis and control women. Only 62 isolates were typed to species level and were subjected to antibiotic susceptibility testing.

### 3.2 Antibiotic Susceptibility Testing

It's performed by vitek-2 method. The antibiotic susceptibility of isolates as appeared in (Fig. 2) was done only for the typable species of CoNs which were 62 strains comprising (50 from patients with infectious mastitis and 12 from control group) which also representing: (*S. epidermidis* 40, *S. hemolyticus* 10, and *S. hominis* 12). It is noticed from (Fig. 1) that Most of CoNS (56/62) isolates were resistant to penicillin G, Cefoxitin, oxacillin, 90.32%(12/62) gentamycin 38% tetracycline 19.35%, Sulfamethoxazole 9.67% (6/62), Ticoplanin 7%, Rifampicin 6.45% (4/62), and Nitrofurantoin (3.3%). While Moxifloxacin, Linezolid, Vancomycin, and Tigecyclin showed full of activity (100%) against different species of the CoNS isolates.

### 3.3 Antibiotic Resistant and *mecA*

The most common resistance pattern appeared in (Fig. 1), Showed that all isolates that showed resistant to different antibiotic by AST Vitek -2 test were positive for detection of *mecA* by PCR technique and staphylococcal chromosome cassette *mec* typing (*SSCmec*) and primers of *mecA* used in PCR technique to evaluate the presence of *mec*A gene. Back to (Fig. 1) the results showed that oxacillin resistant isolates were (56/62) 90.32% cefotaxcim (56/62)) 90.32% Gentamycin Rifampicin and Ticoplanin showed low level (4/62) 6.45% and Erythromycin (24/62) 38.70% Inducible clindamycin resistance and Nitrofurantoin showed low level of resistant (2/62) 3.22%.

Tetracyclin (12/62) 19.35% Trimethoprim-sulfamethoxazole (6/62) 9.67 %While all these isolates collectively including the resistant and the susceptible (62/62) 100%have *mecA* gene.

Distribution of *SCCmec* types in CoNS was determined in all *mec*A isolates. On the principle of the *ccr*B limitation pattern with *HinfI* (the enzyme that cut or restricts the bacterial genome at the site were *ccrB* is located at the following sites according to the type of *SCC* genes (type IV: 264, 227, 154 bp; type III: 537and 106 bp) or with *HinfI / BsmI*227, 171, 153 and 93 bp; type III: 320, 174, 106 and 44 bp).

**Table 1. The number and percentage of CoNS recovered from patients and healthy women**

| CoNS isolates | Mastitis samples (%) | Control samples (% ) |
|---|---|---|
| *S. epidermdis* | 45.45% (30/66) | 45.45% (10/22) |
| *S. haemolyticus* | 15.6% (10/66) | 0 |
| *S. hominis* | 15.6% (10/66) | 9.09% (2/22) |
| *Others* | 24.24% (16/66) | 15.6% (8/22) |
| Total | 100% (66/66) | 100% (22/22) |

*P-value: 0.3186, Non significant at p<0.05*

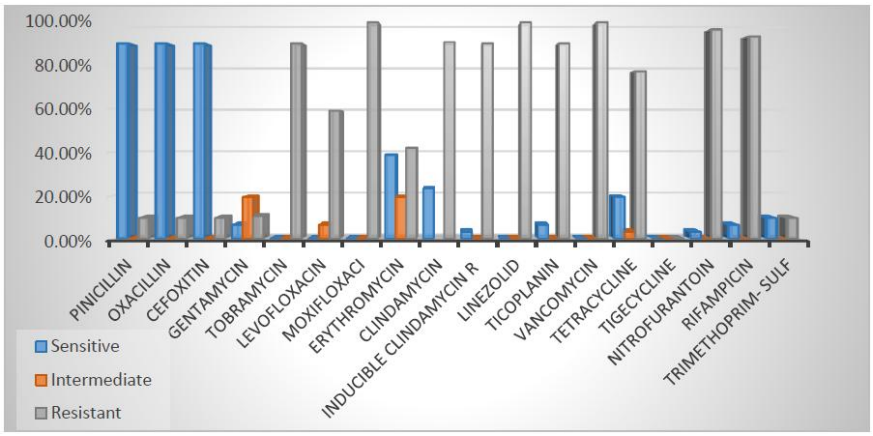


**Fig. 1. Antibiotic sensitivity pattern of 62 coagulase negative staphylococci isolates**

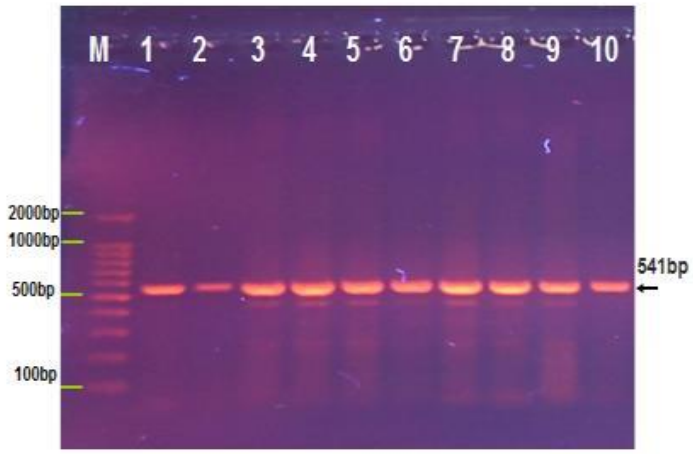


**Fig. 2. Agarose gel electrophoresis image showing the PCR product of methicillin resistance gene (*mecA*) in *Staphylococcus epidermidis* isolates. Where M: Marker (2000-100 bp), lane (1-10) positive Methicillin resistance Gene at (541 bp) PCR product size**

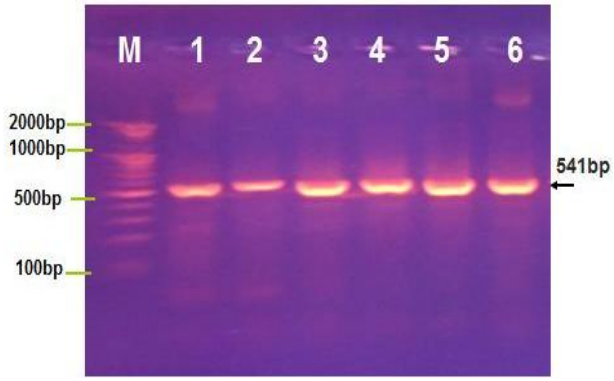


**Fig. 3. Agarose gel electrophoresis image that shown the PCR product of methicillin resistance gene (*mecA*) in *Staphylococcus haemolyticus* isolates. Where M: Marker (2000-100 bp), lane (1-5) positive Methicillin resistance gene at (541 bp) PCR product size**

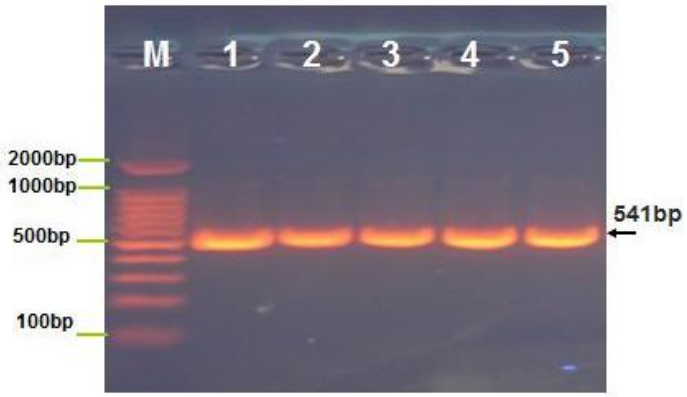


**Fig. 4. Agarose gel electrophoresis image showing the PCR product of methicillin resistance gene *(MecA)* in *Staphylococcus hominis* isolates. Where M: Marker (2000-100bp), lane (1-6) positive methicillin resistance**

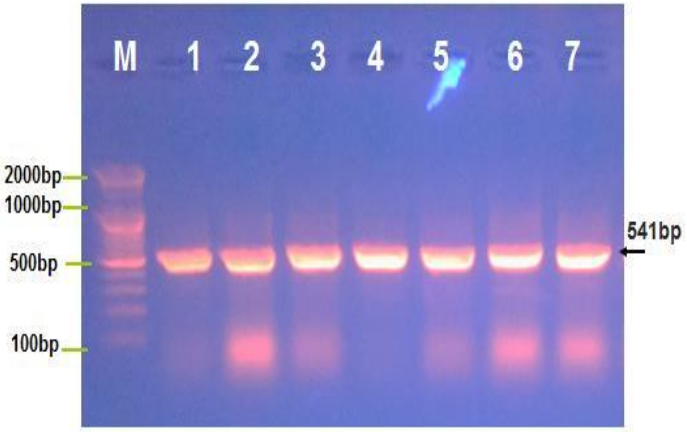


**Fig. 5. Agarose gel electrophoresis image showing the PCR product of methicillin resistance gene (*MecA*) in other coagulase negative isolates. Where M: Marker (2000-100 bp), lane (1-7) positive methicillin resistance gene at (541 bp) PCR product size**

It is clear from Fig. 9 and Table 3 that the distribution of types of *SCC* in different CoNS, the figure revealed that most predominant types between other types of *SCC* was type IV between CoNS isolates 30 (20: *S. epidermidis*, 6: *S. heamolyticus*, 4: *S. hominis*). While type II 8 (6: *S. epidermidis*, 0: *S. heamolyticus*, 2:*S.hominis*) and III appeared 12 (*8: S. epidermidis*, *2: S. heamolyticus, 2: S. hominis*) the 8(*6: S. epidermidis*, 2: *S. heamolyticus*, 2:S.hominis) *mecA* isolates that could not be determine on the principle of their *ccr*B limitation, pattern stay non typable for *SCCmec.*

## 4. DISCUSSION

Antibiotic susceptibility test of CoNS appeared most of CoNS (56/62) isolates were resistant to penicillin G, Cefoxitin, oxacillin, 90.32%. These results are going with the study of 19 who reported that all isolates (100%) of *S. haemolyticus* and *S. hominis* were resistant to oxacillin versus 82.3% of *S. epidermidis* isolates.

Study of [19] found that 86-100% of CoNS isolated from neonatal infections were methicillin- resistant strains (MR-CoNS), moreover, these bacteria was connected with multiple resistance to other antibiotics penicillin and oxacillin resistance was appeared to be 86.8% and 29.7%, at follow but in this research it was 90.32%(56/62).

The main two mechanisms are accountable for the insusceptibility of staph-bacteria to the β-lactam antibiotics the 1st: β-lactamase enzyme production by the CoNS that damage these agents, and the 2nd: change of proteins found in the wall of the bacteria cell named (PBPs) [20].

The resistance of staphylococci to oxacillin the interposed by gene *mec*A which is code to result an additional binding protein for penicillin, PBP2a or 2, which is mentioned either homogeneous or asymmetrically [4]. PBP penicillin binding protein 2a has a small affinity for beta-lactam antibiotic, the homogenous resistance is really recognized with standard test methods, whereas the heterogeneous express is more complex to examination with a few tests, due to only a little part of the PBP2a is expressed in the resistant phenotype.

The high resistance of CoNS to cephalosporin's (90.32%) was attributed to the fact that respectively Cefoxitin is considered to be an excellent inducer of mecA gene expression, therefore, staphylococci resistant to methicillin/oxacillin should be considered resistant to cefoxitin [21]. The result study agreed with the study of [22,23] they reported that the resistance of CoNS to cefoxitin was recorded in 83.3% and 100%.

Antibiotic resistant and *mecA* presence, current research revealed that the phenotypic character of oxacillin-resistance isolates also have *mecA* gene. In current study, 90.32% of *S. epidermidis* isolates carried *mecA* gene which is similar to the study of [24] who reported that 87.5% of *S. epidermidis* isolates harbored *mecA* phenotypically.

[25], also reported that 95.8% of *S. epidermidis* isolates harbored *mecA*. In current study, Table 2. While regarding the genotypic features as shown in PCR that is shown in Figs. 6, 7, 8 and 9. CLSI [26] guidelines indicated that checking for the presence of *mecA* gene by PCR is the most reliable method for detection of MR [27].

It can be suggested that the resistance that was reported may be as a result of determined of the genes responsible for resistant such as *mec*A *mecC*, *ica* gene cluster, *blaZ* (MR) and van A, B, C in charge of vancomycin resistant 5, 28 These genes were however not present in current study with the exception of *mec*A, Figs. 2, 3, 4, 5. Or due to the thickness of the bacterial wall as reported by some author [24].

The results of (Table 3) showed that 80% (34/40) of *S. epidermidis* CoNs that have *mecA* have *ccrB* gene (which is a gene that is responsible for insertion of the resistant genes like *mecA* gene), and *S. haemolyticus* showed the same percentage 80% (8/10), while *S. hominis* showed (52/62) 80% of isolates have *ccrB* gene. This result agreed with result of [25] but disagreed with [28] *ccr* have different type *ccrA, ccrB ,ccrC* and *ccrD ,*so we can suggest that the rest of CoNs isolates that don't showed *ccr*B may have other type of *ccr* genes Figs. 6, 7, 8, 9 [29].

The results in (Fig. 9) is very clear to tell us the distribution of types of *SCCmec* between CoNs isolates. The results mention that type IV is the most predominance between other types which revealed (34/62)54.38% for *S. epidermidis* in comparison with the other types, this result is agreed with [25] *SCCmec* type IV (31%) and III (24.5%), these types are the more widespread that are identified also they found to be more resistant to non-β-lactam antibiotics this result

disagree with [30,31]. It was expected that the elevation of type IV in *S. epidermidis* is because this type is a community acquired and approximately all of the present study species were isolated from community (outpatient) these results are confirmed Employment of full examination described by [32] (10/62) 16.12% *mec*A isolates that cannot be typed on the basis of their restriction pattern stayed without type with complete assay of [33], Fig. 9.

**Table 2. Of *mecA* and anti biotic resistant in CoNS**

| | Antibiotic | Resistant | *mecA* |
|---|---|---|---|
| Pinicillin | P | (56/62)90.32% | (62/62)100% |
| Oxacillin | OX | (56/62)90.32% | (62/62)100% |
| Cefoxitin | CN | (56/62)90.32% | (62/62)100% |
| Gentamycin | GEN | (4/62 ) 6.45 % | (62/62)100% |
| Tobramycin | TO | 0 | (62/62)100% |
| Levofloxacin | LV | 0 | (62/62)100% |
| Moxifloxaci | MO | 0 | (62/62)100% |
| Erythromycin | E | (24/62) 38.70% | (62/62)100% |
| Clindamycin | CY | (2/62) 23.22% | (62/62)100% |
| Clindamycin | ICR | (2/62) 23.22% | (62/62)100% |
| Inducible clindamycin R | LZ | (2/62) 3.22% | (62/62)100% |
| Linezolid | LTI | 0 | (62/62)100% |
| Ticoplanin | VA | (4/62 )6.45% | (62/62)100% |
| Vancomycin | TE | 0 | (62/62)100% |
| Tetracycline | TI | (12/62) 19.35% | 100%(62/62) |
| Tigecycline | F | 0 | 100%(62/62) |
| Nitrofurantoin | RIF | (2/62)3.22 % | 100%(62/62) |
| Monoxycarbolic acid | SXT | 0 | 100%(62/62) |
| Rifampicin | P | (4/62)6.45% | 100%(62/62) |
| Trimethoprim- sulf | OX | (6/62)9.67% | 100%(62/62) |

**Table 3. Staphylococcal cassette chromosome *mec* typing**

| CoNS isolates | *mec* A gene | ccrB gene |
|---|---|---|
| *S. epidermdis* | 40/40 (100%) | 34/40 (85%) |
| *S. haemolyticus* | 10/10 (100%) | 8/10 (80%) |
| *S. hominis* | 12/12 (100%) | 8/12 (80%) |
| Total | 62/62 (100%) | 50/62 (84.09%) |

*P –value: 0.9488, Non-significant at p<0.05*

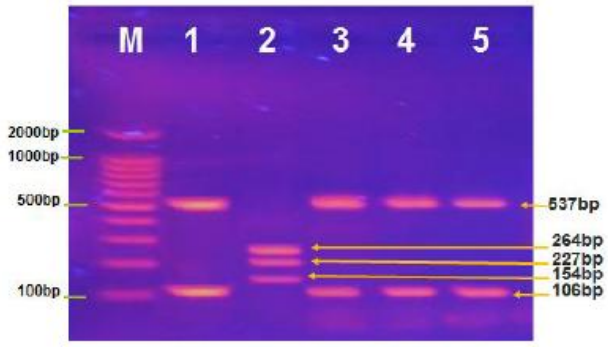


**Fig. 6. Agarose gel electrophoresis image showing the RFLP-PCR product of *ccrB* gene in *Staphylococcus epidermidis* isolates using restriction(1 & 2) positive*SCCmec* genotype (IV) at 264, 227and154bp, lane (3) positive *SCCmec* genotype (III) at 537 and 106bp, and lane (3) positive *SCCmec* genotype (II) at 225 and (106 bp.)**

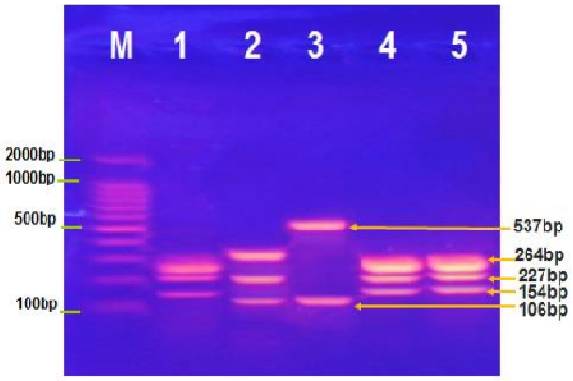


**Fig. 7. Agarose gel electrophoresis image THATt shown the RFLP-PCR product of *ccrB* gene in *Staphylococcus haemolyticus* isolates using restriction enzyme (*HinfI*). Where M: Marker (200-100bp), lane (1, 3, &4) positive *SCCmec SCCmec* genotype (III) at 537 and 106bp. genootype (IV) at 264, 227& 154 bp, and lane (2) positive**

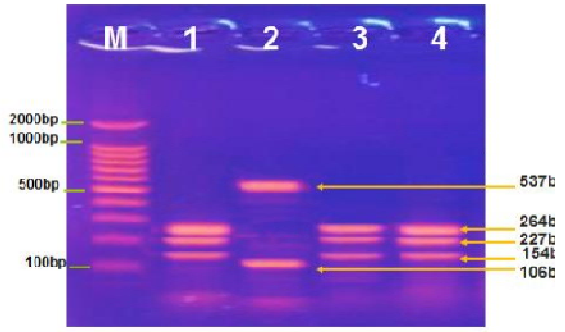


**Fig. 8. Agarose gel electrophoresis image that shown the RFLP-PCR product of *ccrB* gene in *Staphylococcus hominis* isolates using restriction enzyme (*HinfI*). Where M: Marker (2000-100 bp), lane (1,4 &5) positive *SCCmec* genotype (IV) at 264, 227& 154 bp, and lane (3) positive *SCCmec* genotype (III) at 537 and 106bp, and lane (2) positive *SCCmec* genotype (II) at 225 and 106bp.**

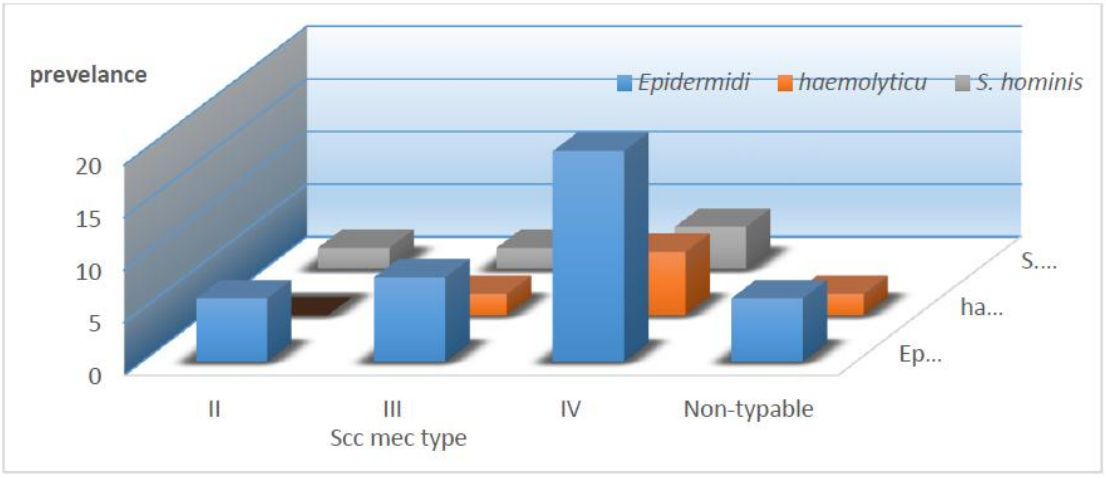


**Fig. 9. Distribution of types of SCCmec genes in CoNS according to the presence of *ccrB***

## 5. CONCLUSION

The result of this study showed that a large percentage of coagulase-negative staphylococci are resistance to methicillin, There is no significant differences between the presence of *mecA* and *ccrB* genes , the prevalence of SCC*mec* type was type III, which encodes the largest number of resistance genes. This information could be used in epidemiological study for preventing of infectious control in hospital and health centers.

## STATISTIC

The statistical analysis was dependent on $x^2$ and represent $P< 0.05$ is significant.

## CONSENT

It is not applicable.

## ETHICAL CONDUCT OF THE STUDY

The study has been conducted in accordance with recommendations guiding obtained from the College of Medicine, Kufa University. The study did not involve biological material or genetically modified organisms. All the isolates involved in the study came from routine samples without any additional materials.

## ACKNOWLEDGEMENTS

The samples of this work was supplied from the Center of breast examination in Al-Sadder hospital in Najaf city –Iraq.Im thank all staff whose help me in this center.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Chambers HF. Methicillin-resistant staphylococci. Clin Microbiol Rev. 1988;1:173–186.
2. Katayama Y, Ito T, Hiramatsu K. Genetic organization of the chromosome region surrounding mecA in clinical staphylococcal strains: role of IS431-mediated mecI deletion in expression of resistancein mecA-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus*. Antimicrob Agents Chemother. 2001;45:1955–1963.
3. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillinresistant *Staphylococcus aureus* N315. Antimicrob Agents Chemother. 1999;43:1449–1458.
4. Hartman BJ, Tomasz A. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus.* Antimicrob Agents Chemother. 1986,29(1): 85-9.
5. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of *Staphylococcus aureus* from itswhole genome: Genomic island SCC. Drug Resist Updat. 2003;6:41–52.
6. Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, Daum RS, Hiramatsu K. Novel type of staphylococcal cassette hromosome mec identified incommunity acquired methicillin-resistant *Staphylococcus aureus* strains Antimicrob Agents Chemother. 2002;46:1147–1152.
7. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, Klang M, Chavalit T, Song JH, Hiramatsu K. Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 1Asian countries: A proposal for a new nomenclature for SCCmec elements. Antimicrob Agents Chemother 2006;50:1001–1012.
8. Ito T, XX Ma, Takeuchi F, Okuma K, Yukawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome mec driven by a novel cassette chromosome recombinase, *ccrC.* Antimicrob. Agents. Chemother. 2004;48:2637–2651.
9. Oliveira DC, Milheiric OC, de Lencastre H. Redefining astructural variant of staphylococcal cassette chromosome, SCCmec type VI. Antimicrob Agents Chemother. 2006;50:3457–3459.
10. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother. 2007;51:264–274.
11. Hisata K, Kuwahara-Arai K, Yamamoto M, Ito T, Nakatomi Y, Cui L, Baba T, Terasawa M, Sotozono C, Kinoshita S,

Yamashiro Y, Hiramatsu K. Issemination of methicillin-resistant staphylococci among healthy Japanese children. J. Clin. Microbiol. 2005;43:3364–3372.

12. Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL. Related clones containing SCCmec type IV predominate among clinically significant Staphylococcus epidermidis isolates. Antimicrob Agents Chemother. 2003;47:3574–3579.
13. Hiramatsu K, Watanabe S, Takeuchi F, Ito T, Baba T. Genetic characterization of methicillin-resistant *Staphylococcus aureus.* Vaccine. 2004;22(Suppl. 1):S5–S.
14. Lactational culture protocol page 1-3.
15. Delgado S, Rebeca Arroyo, Esther Jiménez, Maria L Marín, Rosa delCampo, Leonides Fernández, Juan M Odríguez. *Staphylococcus epidermidis* strains isolated from breast milk of women suffering infectious mastitis: potential virulence traits and resistance to antibiotics. BMC Microbiology. 2009;9:82.
16. McFadden A, Toole G. Exploring women's views of breastfeeding: a focus group study within an area with high levels of socio-economic deprivation. Maternal & Child Nutrition. 2000;2:156-168.
17. Jeanesse Scerri Stefan Monecke, Michael A. Borg. Prevalence and characteristics of community carriage of methicillin-resistant *Staphylococcus aureus* in Malta Journal of Epidemiology and Global Health. 2013;3:165–173.
18. Yang JA, Park DW, Sohn JW, Kim MJ. Novel PCR restriction fragment length polymorphism analysis for rapid typing of staphylococcal cassette chromosome mec elements. J. Clin Microbiol. 2006;44:236–238.
19. Abd El Hafez M, Khalaf NG, El Ahmady M, Abd El Aziz A, Hashim AG. An outbreak of methicillin resistant *Staphylococcus epidermidis* among neonates in a hospital in Saudi Arabia. J Infect Dev Ctries. 2011;5:692-699.
20. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, et al. Methicillin-resistant *Staphylococcus aureus:* a consensus review of the icrobiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med. 1993;94(3):313-28.
21. McKinney TK, Sharma VK, Caig WA, Archer GL. quot;. Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus* (mecA) is corepressed but not co-induced by cognate mecA and beta-lactamase regulators & quot. J. Bacteriol. 2001;183:6862–868.
22. Seccchi C, Antunes AL, Perez LRR, Cantarelli, D'Azevedo PA. Identification and detection of Methicillin resistance in non-epidermidis coagulase-negative staphylococci. Brazilian Journal of Infectious Diseases. 2008;12(4):316-320.
23. Ustulin DR, Cunha MLRS. Methods for detection of oxacillin resistance among coagulase-negative staphylococci recovered from patients with bloodstream infections at the University Hospital in Brazil. Journal of Virology and Microbiology; 2012.
24. Tena D, Beatriz López-Garrido, Cristina Losa. Clinical mastitis in breastfeeding women: Study of 56 cases. Jornal Infectious Diseases. DOI: 10.1080/23744235.2016.1204662.
25. Rohde H, Burdelski C, Bartscht K, Hussain M, Buck F, Horstkotte MA, Knobloch JK, Heilmann C, Herrmann M, Mack D. Induction of Staphylococcus *epidermidis* biofilm formation via proteolytical processing of the accumulation associated protein by staphylococcal and host protease. Mol Microbiol. 2005;55:1883-1895.
26. Pourmand MR, Abdossamadi Z, Salari MH, Hosseini M. Slime layer formation and the prevalence of mecA and aap genes in *Staphylococcus epidermidis* isolates. J Infect Dev Ctries. 2011;5(1):34–40.
27. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests, 9th ed. Wayne, PA: CLSI; 2006.
28. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol. 2002;292:107-113.
29. Kim MN, Pail H, Woo JH, Ryu JS, Hamamatsu K. Vancomycin-intermediate *Staphylococcus aureus* in Korea. Journal of Clinical Microbiology. 2000;38:3879-3881.
30. Swenson JM, Tenove FC, The cefoxitin disk study group. Results of disk diffusion testing with cefoxitin correlate with presence of mecA in *Staphylococcus* spp. J. Clin. Microbiol; 2005.
31. Ghosh A, Yogesh Singh, Arti Kapil, Benu Dhawan. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing of

clinical isolates of coagulase-negative staphylocci (CoNS) from a tertiary carehospital in New Delhi, India. 2016;143:365-370.

32. Arabestani MR, Mohammad Reza Arabestani, Mohammad Yousef Alikhani, Manoochehr Karami, Elham Salim Ghale. Prevalence of coagulase-negative staphylococci and determination of antimicrobial resistance in accompany with types of SCCmec in isolated of nosocomial infections Student Tehran University Medical Journal. 2016;73(12):888-894.

33. Zhang K, McClure JA, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2009;53: 531–540.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://sciencedomain.org/review-history/22391*