Scientific Research Publishing

American Journal of Molecular Biology, 2022, 12, 122-133
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Diversity and Distribution of Staphylococcal Chromosomal Cassettes Mec (SCCmec) Types I, II and III in Coagulase-Negative Staphylococcal Strains Isolated from Surfaces and Medico-Technical Materials of the University Hospital of Abomey-Calavi/Sô-Ava

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How to cite this paper: Chimene, N., Fresnel, C., Gustave, D., Haziz, S., Akim, S., Aurel, D., Enorck, K., Farid, B., Clément, A. and Saïd, B.-M.L. (2022) Diversity and Distribution of Staphylococcal Chromosomal Cassettes Mec (SCCmec) Types I, II and III in Coagulase-Negative Staphylococcal Strains Isolated from Surfaces and Medico-Technical Materials of the University Hospital of Abomey-Calavi/Sô-Ava. *American Journal of Molecular Biology*, **12**, 122-133.
https://doi.org/10.4236/ajmb.2022.123011

Received: April 21, 2022
Accepted: July 3, 2022
Published: July 6, 2022

Abstract

The coagulase-negative staphylococci (CoNS) have long been considered to be low pathogenicity. The possibility of a horizontal transfer of resistance and virulence genes from *S. aureus* to CoNS could increase the pathogenicity of these bacteria. The objective of this work is to contribute to a better knowledge of the pathogenicity of (CoNS) strains isolated from surfaces and medico-technical materials of the University Hospital of Abomey-Calavi/Sô-Ava. Seventy strains of CoNS isolated from surfaces and medico-technical materials of the University Hospital of Abomey-Calavi were tested for methicillin resistance. The resistance to methicillin was evaluated phenotypically by the resistance of the strains to cefoxitin and then confirmed by the search for the *mecA* gene using PCR. The genes encoding staphylococcal chromosomal cassette (SCCmec) types I, II and III originally found in *S. aureus* were tested in CoNS by multiplex PCR using specific primers. All the strains studied

showed resistance to methicillin. However, only 28.5% (20/70) carried the mecA gene. SCCmec was identified in only 17.14% (12/70) of these strains. Four strains carried *mecA* gene as well as one of the three types of SCCmec searched. SCCmec types I, II and III were identified in CoNS strains studied. SCCmec type I was the most frequent chromosomal cassette in $mecA^+$ strains, only or in association with another SCCmec. The study also revealed methicillin-resistant strains carrying SCCmec lacking the mecA gene. Finally, 60% (12/20) of the strains were found to be non-typeable. Our results show that CoNS strains present a high resistance to methicillin and the source of this resistance in the CoNS of our study is not only the mecA gene. There is also a high diversity of SCCmec, justified by a large number of non-typeable CoNS strains. The $mecA^-$ $SCCmec^+$ methicillin-resistant strains deserve to be sequenced for further studies.

Keywords

Coagulase Negative Staphylococci, *mecA,* Staphylococcal Chromosomal Cassette

1. Introduction

The coagulase-negative staphylococci (CoNS) have long been considered to be low in pathogenicity, but are increasingly implicated in many healthcare-associated infections [1]. If nowadays, these bacteria occupy an important place in human pathology [2] it's because, since their appearance, they have constantly developed resistance mechanisms to antibiotics in order to ensure their survival [3]. This resistance is linked to their great genomic plasticity which can be acquired or contributed by a plasmid or other mobile genetic elements such as staphylococcal chromosomal cassettes, transposons, bacteriophages or insertion sequences [4]. Thus, after the emergence of methicillin-resistant S. aureus strains, a consequence of the excessive use of this antibiotic; we observe the appearance of coagulase-negative staphylococci resistant to methicillin (MRCoNS), an antibiotic of choice used in the staphylococcal infection's treatment [5]. However, in *S. aureus* the resistance to methicillin (MRSA) leads to resistance to almost all beta-lactam antibiotics [6]. This makes the management of patients a challenge for clinicians.

The main mechanism of the resistance to methicillin is related to beta-lactam target modification [6] [7]. Resistance to this semi-synthetic antibiotic is under the control of the mecA gene located in a mobile genetic element called the staphylococcal chromosome cassette mec (SCCmec) [8]. SCCmec harbors the mec genes, which confer resistance not only to methicillin but also to almost all other beta-lactams [9]. SCCmec has two components, it is about: the mec gene complex and the cassette chromosome recombinase (ccr) gene complex. The mec gene complex which consists of mecA, the regulatory genes, and the associated insertion sequences; has been classified into six different classes (A, B, C1, C2, D,

and E) together with cassette chromosome recombinase (ccr) genes (ccrC or the pair of ccrA and ccrB) encoding recombinases that mediate the integration and excision of SCCmec into and from the chromosome.

Different types of SCCmec have been identified in staphylococci. Among them, SCCmec I, II and III are more particularly present in Hospital associated MRSA strains (HA-MRSA), while new SCCmec allelic variants, types IV to VIII, have been identified in Community associated MRSA strains (CA-MRSA) [10]. This implies that coagulase-positive and coagulase-negative strains of staphylococci exchange staphylococcal chromosomal cassettesmec (SCCmec) by horizontal transfer, thus promoting the acquisition of antibiotic resistance genes by the latter [11].

The aim of this study is therefore to investigate the diversity and distribution of staphylococcal chromosome cassettes mec (CCSmec) types I, II and III in coagulase-negative staphylococcal strains isolated from surfaces and medico-technical materials from the University Hospital of Abomey-Calavi/Sô-Ava (South Benin) from January to June 2019 in the Departments of Neonatology, Pediatrics, Maternity Ward, Operating Room and Central Sterilization.

2. Materials and Methods

2.1. Collection of Bacterial Isolates

Seventy strains of coagulase-negative staphylococci were previously collected from surfaces and medico-technical materials at the Abomey-Calavi/Sô-Ava University Hospital and identified phenotypically by the API STAPH galleries [12]. These 70 strains represent 10 species, namely *S. xylosus* (n = 12), *S. capitis* (n = 10), *S. sciuri* (n = 10), *S. hominis* (n = 10), *S. cohnii* (n = 8), *S. epidermidis* (n = 6), *S. lugdunensis* (n = 6), *S. haemolyticus* (n = 4), *S. lentus* (n = 2), and *S. schleifeiri* (n = 2).

2.2. Susceptibility of CoNS Strains to Methicillin

The susceptibility of the CoNS isolate was investigated by cefoxitin disk diffusion method on the Muller Hinton agar medium according to the EUCAST [13]. The bacterial suspension was standardized using the 0.5 McFarland control.

2.3. DNA Extraction

DNA extraction of the different SCN strains was done according to the method adapted from Rasmussen and Morrissey [14] [15]: after inoculating a colony of the strain to be tested on MH agar medium poured into petri dishes, the dishes were incubated at 37˚C for 18 h. Then, the preculture of the different colonies obtained on the petri dishes was done in 1 mL of liquid MH in an eppendorf tube. The tubes were incubated at 37˚C for 18 h. The obtained bacterial cultures were centrifuged at 12,000 rpm for 5 minutes. The supernatant was poured off and 500 µL of sterile distilled water was added to the bacterial pellet and heated in a dry bath at 95˚C for 15 minutes. Then, the heated pellet was centrifuged at

12,000 rpm for 5 minutes. The resulting supernatant was emptied into another eppendorf tube and 500 µL of 70% (v/v) alcohol was added. Centrifugation was performed again at 12000 rpm for 5 minutes. The resulting supernatant was again emptied and the eppendorf tubes were left for 24 hours in a laminar flow hood for drying. The DNA pellets were suspended in 50 µL of EDS and stored at 4˚C for immediate use or at −20˚C for long-term storage.

2.4. mecA Gene Detection

The detection of the *mecA* gene in methicillin-resistant CoNS (MR-CoNS) strains was performed by monoplex PCR using specific primers (Table 1). The reaction mixture used for the amplification of this gene was composed of 2 µL of buffer, 0.1 µL of Taq polymerase at a final concentration of 0.025 units/µL, 0.2 µL of $MgCl_2$, 0.4 µL of dNTP, 1 µL of reverse and forward primer of the *mecA* gene at a final concentration of 10 µM, 5 µL of DNA and 8.3 µL of sterile distilled water for a final volume of 20 µL. The amplification program used consisted of an initial denaturation at 94˚C for 3 min followed by 36 cycles of denaturation at 94˚C for 1 min and 30 s, hybridization at 55˚C for 1 min, elongation at 72˚C for 1 min, final elongation at 72˚C for 10 min and refrigeration of the amplified product to 10˚C.

2.5. SCCmec Typing

The typing of CCSmec present in CoNSRM was done via multiplex PCR using specific primer pairs (Table 1) capable of detecting CCSmec type I, II and III. The sequences of the different primers used are shown in Table 1. The reaction mixture consisted of 2 µL of buffer, 0.1 µL of Taq polymerase at a final concentration of 0.025 units/µL, 0.4 µL of dNTP, 1 µL at a final concentration of 10 µM of the sense and antisense primers of each of the three primer pairs, 5 µL of DNA and 6.5 µL of sterile distilled water for a final volume of 20 µL. The amplification program used was as follows: initial denaturation at 94˚C for 3 min followed

Table 1. List of primers for mecA gene detection and SCCmec typing.

Target	Name/ Primers	Sequences (5' → 3')	Amplified fragment Size (bp)	SCCmec Type
SCCmec	CIF2 F	TTCGAGTTGCTGATGAAGAAGG	494	I
	CIF2 R	ATTTACCACAAGGACTACCAGC		
	KDP F1	AATCATCTGCCATTGGTGATGC	284	II
	KDP R1	CGAATGAAGTGAAAGAAAGTGG		
	RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	III
	RIF5 R13	GTCACAGTAATTCCATCAATGC		
mecA	mecA F	CCAGGAATGCAGAAAGACC	675	
	mecA R	TCACCTGTTTGAGGGTGGAT		

by 36 cycles of denaturation at 94˚C for 1 min and 30 s, hybridization at 57˚C for 1 min, elongation at 72˚C for 1 min, final elongation at 72˚C for 10 min and cooling of the amplified product to 10˚C.

2.6. Data Analysis

The Excel 2019 workbook was used to make graphs to better analyze data. The ± signs were used to reflect the presence or absence of the desired gene and staphylococcal chromosomal cassettes.

3. Results

3.1. Susceptibility of CoNS Strains to Methicillin

A total of 70 CoNS strains isolated from surfaces and medical equipment at the Abomey-Calavi/Sô-Ava University Hospital were enrolled in this study. The susceptibility to methicillin of CoNS strains revealed that all (100%) strains studied were resistant to the used antibiotic (Figure 1). Thus, among the strains resistant to methicillin, *S. xylosus* is the most represented species, representing 17.14% of all MR-CoNS strains. *S. lentus* and *S. schleifeiri* strains were weakly represented in methicillin-resistant species (2.85% each). *S. capitis*, *S. sciuri*, *S. hominis*, *S. epidermidis*, *S. lugdunensis* and *S. haemolyticus* are resistant in varying proportions.

3.2. *mecA* Gene Detection

The detection of the *mecA* gene in all seventy methicillin-resistant CoNS strains was performed by monoplex PCR. Our results show that 28.5% (20/70) of the CoNS strains possess the searched gene (Figure 2). Thus, *S. xylosus* (8/12), *S.*

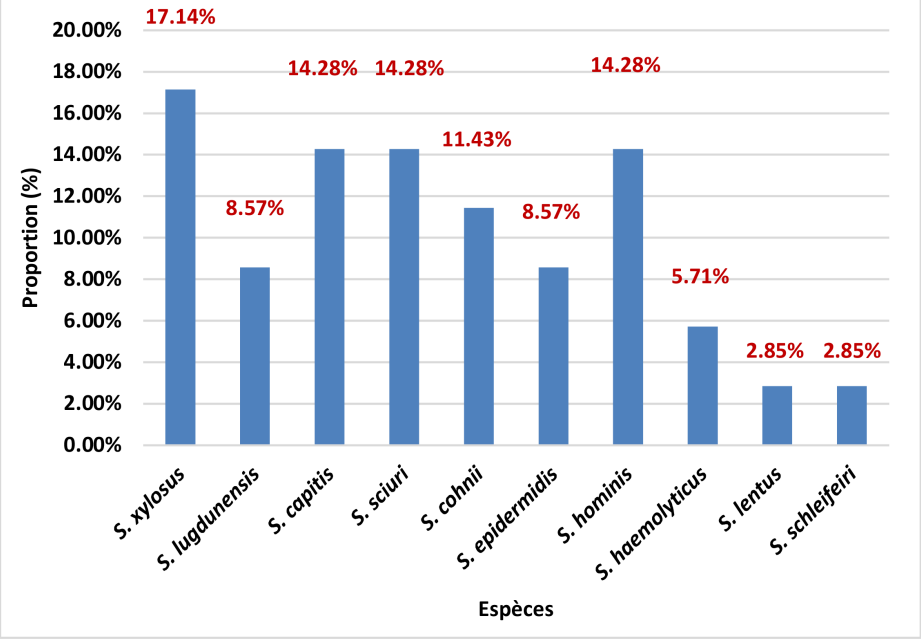

Figure 1. Methicillin resistance rate of CoNS species previously identified.

lugdunensis (2/6), *S. capitis* (2/10), *S. sciuri* (2/10), *S. lentus* (2/2), *S. haemolyticus* (2/4) and *S. epidermidis* (2/6) possess the *mecA* gene. Inside the MR-CoNS species studied, the *mecA* gene is most present in *S. xylosus* strains (40%).

3.3. SCCmec Typing

To assess the distribution of SCCmec in CoNS, Multiplex PCR was done. Analysis revealed the presence of one of the three SCCmec in twelve (17.14%) of the seventy CoNS strains studied (Figure 3, Table 2). Among the *mecA*$^{+}$ strains, four (40%) were found to carry one of the three SCCmec. Thus, SCCmec types I, II and III alone or in combination with another SCCmec were distributed among twelve CoNS strains. However, it was observed that among the strains carrying at least one of the three SCCmec type, two CoNS strains (*S. cohnii* and *S. sciuri*) carry SCCmec lacking the *mecA* gene. Our results also show that SCCmec type I, either alone or in combination, is the most frequent SCCmec in the CoNS strains studied. Indeed, it was identified alone and in combination with SCCmec type III (n = 4). The other type of SCCmec identified was SCCmec type II and it was identified as many times as SCCmec type III. This study also shows a high genetic diversity of SCCmec within the MR-CoNS since 60% of

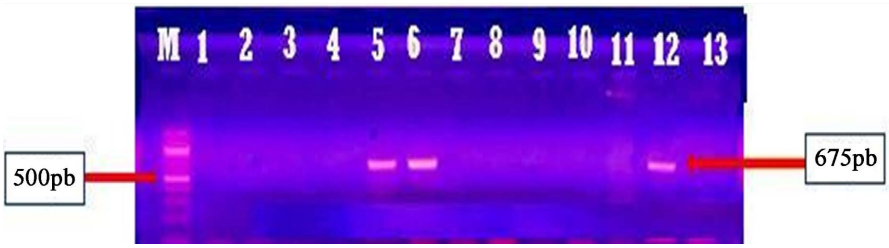

Figure 2. Agarose gel electrophoresis for the PCR detection of *mecA* gene in CoNS species. Lane M: 100-bp molecular weight marker. Lane 1, 2, 3, 4, 7, 8, 9, 10, 11, and 13: CoNS isolates lacking *mecA* gene. Lane 5, 6 and 12: CoNS isolates positive for *mecA* gene.

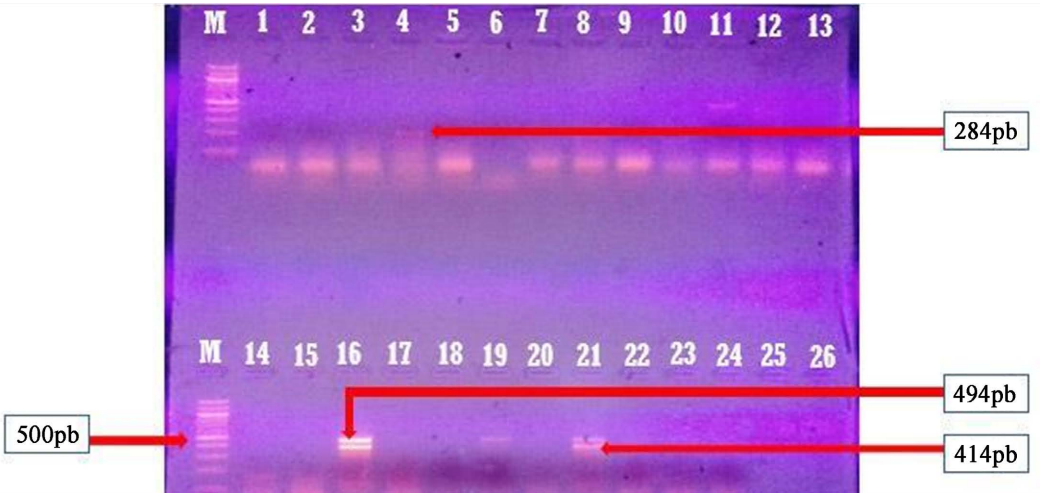

Figure 3. Distribution of SCCmec in methicillin-resistant CoNS species. Lane M: 100-bp molecular weight marker. The presence of the 494, 284 and 284 bp bands reveals the existence of the genes coding for SCCmec types I, II and III respectively.

Table 2. mecA gene and SCCmec repartition in CoNS species.

Genes	*S. xylosus*	*S. lentus*	*S. cohnii*	*S. hominis*	*S. schleifeiri*	*S. lugdunensis*	*S. capitis*	*S. sciuri*	*S. haemolyticus*	*S. epidermidis*
mecA	8	2	-	-	-	2	2	2	2	2
SCCmec I	1	-	-	-	-	-	-	1	-	-
SCCmec II	1	-	1	-	-	-	-	-	-	-
SCCmec III	-	-	-	-	-	-	-	-	-	-
SCCmec I + III	-	-	-	-	-	-	1	-	-	1
Non-typeables	10	2	7	10	2	2	9	9	4	5
Total	12	2	8	10	2	6	10	10	4	6

mecA$^+$ strains could not be typed (Figure 3, Table 2).

4. Discussion

CoNS are ubiquitous bacteria with a strong adaptive capacity and are among the most common germs isolated in hospitals. For a long time considered not very pathogenic, they are, as shown by recent studies, more and more incriminated in health care associated infections [14]. The increase in antibiotic-resistant strains of CoNS increases the pathogenicity of these strains and complicates the treatment of patients with infections caused by these bacteria [15]. This study therefore focused on the determinism of CoNS strains carrying the *mecA* gene and its genetic carrier SCCmec. It was found that all CoNS strains in this study were resistant to methicillin when tested on MH agar medium. Results from many other studies have shown that the antibiotic resistance rate of CoNS to methicillin is greater than 50% [16] [17]. However, other studies have shown that the resistance rate is much lower, below 50% [15] [18]. Also, the MRCoNS strains that are resistant to the beta-lactam class antibiotics are also susceptible to showing resistance to another class of antibiotics. Indeed, the gene coding for methicillin resistance is carried by the SCCmec, a mobile genetic element that can carry resistance genes to other antibiotics [19]. In the study made by Socohou and al., CoNS strains with methicillin resistance showed resistance to other antibiotics such as fosfomycin, erythromycin and vancomycin [13]. Bhowmik and al. showed in their study that methicillin-resistant *S. aureus* strains also show resistance to other antibiotics such as clindamycin, linezolid or erythromycin and that this antibiotic resistance is a function of the SCCmec carried by *S. aureus* strains [20]. In our study, *S. xylosus* is the species most represented among the methicillin-resistant CoNS strains. It constitutes 17.14% of the MRCoNS species studied at the expense of *S. epidermidis, S. capitis, S. hominis, S. lugdunensis.* This unusual rather finding may be a function of the origin and location of sampling as well as the size of the samples [15] [20] [21].

It is also noted that not all CoNS strains with methicillin resistance have the

mecA gene. Indeed, only 20 (28.5%) of 70 methicillin-resistant strains carried the researched gene, a percentage that is much higher than that recorded by Xu *et al.* [20]. This result could be explained by the heterogeneity of the expression of methicillin resistance in CoNS strains. In addition, several factors are susceptible to influence the expression of this resistance in these strains [22] [23]. In fact, Ph, temperature and salt concentration can affect the expression of methicillin resistance in CoNS strains. In addition, there are other mechanisms of methicillin resistance in coagulase-negative staphylococci [24]. Also, according to the CNRS CoNS strains with true methicillin resistance and with the *mecA* gene in their genome are quite rare [25]. Although having high scores, methods for phenotypic detection of methicillin resistance would not be as reliable as genotypic methods for detecting resistance to this class of antibiotics. In this regard, the work of Asante *et al.* shows that 05 strains of CoNS with the *mecA* gene were not detected as *mecA*$^{+}$ after their resistance to methicillin was assessed phenotypically [16].

SCCmec are mobile genetic elements and also genetic carriers the *mecA* gene, the gene governing methicillin resistance. Several types of SCCmec have been identified among the staphylococcal strains responsible for healthcare-associated infections. Thus, in this study, type I, II and III SCCmec were detected in only 12 CoNS strains (17.14%) with cefoxitin resistance and in 8 CoNS strains (40%) with the *mecA* gene in their genome. The three types of SCCmec investigated in this study are the types of staphylococcal chromosomal cassette most often identified in staphylococcal strains responsible of healthcare associated infections and therefore logically predominate over other SCCmec such as SCCmec types IV, V, VI, VII and VIII often found in cases responsible for the community associated infections [25] [26]. Our results show that in this study there are fewer CoNS strains carrying the *mecA* gene as well as one of the SCCmec types I, II and III than CoNS strains carrying the *mecA* gene and not typable for one of the three SCCmec types searched (60%). This shows that the SCCmec types I, II and III previously frequently identified in MRCoNS strains are being replaced by the smaller SCCmec types IV and V. Other studies have found similar results to ours in that the most frequent SCCmec identified in MRCoNS strains were not type I, II or III [18] [20] [27] [28]. The current preponderance of type IV, V, VI, VII and VIII SCCmec in hospital associated MRCoNS strains can be explained by the small size of these chromosomal cassettes. Indeed, their small size favors their displacement and transmission from one strain of CoNS to another. Furthermore, the great diversity of SCCmec within CoNS strains would also explain the fact that not all the strains studied could be typed. Then, they could carry one of the other SCCmec identified to date [29].

The identification of CoNS strains possessing SCCmec and lacking the *mecA* gene in their genome would mean that these strains may have lost the researched gene. Indeed, although SCCmec are generally stable once within a staphylococcal strain, they can be damaged if certain conditions are met. For example, there

have been cases where SCCmec lack the *mecA* gene. This may be caused by long storage of CoNS strains at low temperature (−80˚C). Similar results were obtained in other studies where *S. aureus* strains lost the *mecA* gene complex after long storage of these strains at low temperature [30] [31]. In such cases, the SCCmec although retained within the CoNS strain would lack the *mecA* gene and this strain would be *mecA*$^-$ after PCR although carrying a SCCmec. This may be the case in this study as the strains studied were stored at low temperature for a significant period of time.

In view of the significant number of CoNS with the methicillin resistance gene and staphylococcal chromosomal cassette in this study, it's clear that this group of bacteria shouldn't be neglected in daily practice as multidrug resistance is a major constraint in the treatment of infections caused by these bacteria. It's therefore necessary not only to carry out more in-depth studies on this bacterial group but also to adopt measures to improve hygiene in hospitals in order to limit possible contamination that could lead to infections.

5. Conclusion

Often neglected because their pathogenicity is much weaker than that of *S. aureus* strains, CoNS are nowadays incriminated in many cases of nosocomial infections. Our results show that CoNS strains present a high resistance to methicillin and the source of this resistance in the CoNS of our study is not only the *mecA* gene. It's therefore essential to evaluate their antibiotic susceptibility profiles and to determine the source of their resistance to these antibiotics in order to prescribe appropriate antibiotic therapy. This study also shows a high diversity of SCCmec in CoNS strains isolated from surfaces and medico-technical materials of the University Hospital of Abomey-Calavi/Sô-Ava (South Benin), as shown by the large number of non-typeable CoNS strains. The *mecA*$^-$ *SCCmec*$^+$ meticillin resistant strains deserve to be sequenced for further studies. All three types of SCCmec sought were identified in this study but a large number of *mecA*$^+$ CoNS strains could not be typed for the I, II and III SCCmec. Then, these strains should be typed for one of the thirteen other SCCmec identified to date. These staphylococcal chromosomal cassettes, which carry the *mecA* gene, may also be the genetic carriers of many other antibiotic resistance genes. The mobility of these genetic elements could then favor their movement between the different CoNS strains by horizontal transfer, a hypothesis that was verified since one of the SCCmec studied was detected in the CoNS strains. More attention should also be paid to CoNS, their antibiotic resistance profile, the genes that mediate antibiotic resistance and their genetic backbone. Meticillin-resistant strains with *mecA*$^-$ SCC*mec*$^+$ genotype deserve to be sequenced for further study. We also plan to search for other types of staphylococcal chromosome cassette in non-typeable CoNS. On the other hand, whole genome sequencing will help to understand the mechanism of acquisition of the mecA gene in strains that do not carry the staphylococcal chromosome cassette mec.

Acknowledgements

We thank all the staff of the Laboratory of Biology and Molecular Typing in Microbiology of University of Abomey-Calavi, Benin and the laboratory of Molecular Biology and Bioinformatics Applied to Genomics of National University of Sciences, technologies, engineering and mathematics of Abomey, Benin for their help and collaboration in the development of this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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