Diversity of antibiotic resistance genes and staphylococcal cassette chromosome mec elements in faecal isolates of coagulase-negative staphylococci from Nigeria

Luca Agostino Vitali1, Dezemona Petrelli2, Adebayo Lamikanra3, Manuela Prenna2 and Ezekiel Olugbenga Akinkunmi1,3*

Abstract

Background: Coagulase-negative staphylococci (CoNS) are opportunistic pathogens found as colonisers of the human gut. This study was carried out to examine the genetic resistance mechanisms in faecal isolates of CoNS. The study investigated 53 non-duplicate CoNS isolates obtained from the fresh stool samples of apparently healthy subjects in the community of Ile-Ife, South-Western Nigeria. Antibiotic susceptibility testing was assessed by the disc diffusion test while antibiotic resistance genes were analysed by PCR. mecA positive isolates were analysed by Staphylococcal Chromosome Cassette mec (SCCmec) and cassette chromosome recombinase (ccr) complex typing methods.

Results: Resistance genes were detected only in isolates that showed resistance by phenotypic screening. The aac(6′)–aph(2″) gene was detected in all the three isolates resistant to gentamicin. Four of the five erythromycin resistant isolates were positive for the ermC gene, the remaining isolate carried the msrA gene. The tetK gene was detected in 6 of the 7 tetracycline resistant isolates while 4 possessed the tetM gene. Three of the isolates (S. haemolyticus, S. xylosus and S. capitis) had both genes. Several SCCmec types were found: SCCmec I- ccrABβ2-α2 (4 isolates: 3 S. epidermidis, 1 S. warneri), SCCmecIVb- ccrAB2-α3 (1 isolate: S. epidermidis), SCCmecIVd- ccrABβ2-α3 (8 isolates: 3 S. epidermidis, 2 S. xylosus, 1 S. saprophyticus, 1 S. warneri, 1 S. capitis), and untypeable (2 isolates: S. epidermidis).

Conclusion: This genetic background could be a reservoir for interspecies gene transfer among CoNS and S. aureus in the intestinal tract.

Keywords: Coagulase negative staphylococci, Methicillin resistance, Antibiotic resistance genes, Gastrointestinal tract

Background

Coagulase-negative staphylococci (CoNS) are opportunistic pathogens commonly associated with nosocomial infections [1]. Most CoNS strains have been reported to have acquired resistance to methicillin and almost all classes of antimicrobial agents [2,3]. The high resistance rates among CoNS have reduced the ability of health care to treat infections associated with them and led to a prolonged course of infections with severe consequences.

In the vast majority of staphylococcal isolates, resistance to macrolides such as erythromycin has been reported to be due to N6-dimethylation of a 23S rRNA adenine residue preventing macrolide binding to the 50S ribosomal subunits. In the hospital setting, clinical isolates possessing the erm(A) and/or erm(C) gene coding for rRNA methylases were isolated more frequently than erm(B) positive ones [4]. The expression of methylases is usually induced by the presence of 14- or 15-membered macrolides via a translational attenuation mechanism. Modification by mutation of the translation attenuation region may lead to constitutive expression of the methylases even in the absence of inducer macrolides [5]. When expressed, methylases also confer cross-resistance

* Correspondence: eoakinmi@oauife.edu.ng
1 School of Pharmacy, University of Camerino, Camerino, Italy
2 Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria
Full list of author information is available at the end of the article

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to lincosamides and to streptogramin B compounds (MLS\textsubscript{B} phenotype). Also point mutations, matching within the sequence of the domain V of the 23S rRNA or in the proteins of the large ribosomal subunit L4 and L22 may be responsible for MLS\textsubscript{B} resistance [5]. For full resistance to the streptogramine combination quinupristin-dalfopristin, strains need to carry additional resistance to streptogramin A compounds, which may be mediated by acetylation (acetyl transferase genes vat\textit{A}(A), vat\textit{B}(B) and vat\textit{C}(C), or by putative efflux pumps encoded by \textit{vga}(A) and \textit{vga}(B) [5,6].

Tetracycline resistance in staphylococci is either based on the expression of a ribosomal protection factor encoded by the widely disseminated \textit{tet}(\textit{M}) gene or mediated by \textit{tet}(\textit{K}) mediated efflux of the antibiotics [7]. For aminoglycoside resistance, the presence of aminoglycoside modifying enzyme genes \textit{aac}(6\′)-\textit{aph} (2\′), \textit{aph}(3\′)-\textit{Ila} and \textit{ant}(4\′)-\textit{Ia} has been analysed. The most frequently encountered gene in staphylococci is the \textit{aac}(6\′)-\textit{aph}(2\′) which codes for a bifunctional enzyme and confers resistance to gentamicin, tobramycin, kanamycin and when over-expressed to amikacin but not to streptomycin [8]. For the quinolones such as ciprofloxacin and pefloxacin, a main mechanism of resistance is the spontaneous accumulation of mutations in the genes encoding subunits of the DNA gyrase (\textit{gyrA} and \textit{parC}) [9]. Other important antimicrobials include chloramphenicol and co-trimoxazole (trimethoprim + sulphamethoxazole). Resistance to chloramphenicol is mainly mediated by the \textit{catA} gene which is responsible for the chloramphenicol acetyl transferase while co-trimoxazole resistance is due to mutations of the enzyme dihydrofolate reductase encoded by the \textit{dhfr} gene [10].

Methicillin resistance in staphylococci is mainly due to the expression of the \textit{mecA} gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for \(\beta\)-lactams [11]. \textit{mecA} is located on a 21-to 67-kb mobile genetic element (MGE) called Staphylococcal Chromosome Cassette meca (SCC\textit{mec}) [11,12].

Different SCC\textit{mec} elements in staphylococci have been classified and characterized according to the combination of two parts: the \textit{ccr} complex and the \textit{mec} complex. Cassette chromosome recombinase (\textit{ccr}) genes (\textit{ccrC} or the pair of \textit{ccrA} and \textit{ccrB}) encode recombinases that mediate integration and excision of SCC\textit{mec} into and from the chromosome [12-14]. The \textit{ccr} gene(s) form the \textit{ccr} gene complex. The \textit{mec} gene complex on the other hand, consists of \textit{mecA}, \textit{mecR1} and \textit{mecI} regulatory genes and associated insertion sequences and has been classified into six different classes: A, B, C1, C2, D and E [13,14]. The regions located between these complexes are called \textit{J} (joining) regions. In every SCC\textit{mec} elements there are three of these regions (I1-I3) and polymorphisms in the regions are used for the definition of SCC\textit{mec} type IV subtypes [15]. In addition to \textit{ccr} and \textit{mec} gene complexes and \textit{J} regions, SCC\textit{mec} contains a few other genes or pseudogenes that does not appear to be essential to the bacterial cell with exceptions including various other MGE, e.g. insertion sequences, transposons and integrated plasmids mediating resistance to non-\(\beta\)-lactam antibiotics or heavy metals [12].

Based on the classes of the \textit{mec} gene complex and the \textit{ccr} gene types, eleven types (I to XI) of SCC\textit{mec} have been assigned for \textit{Staphylococcus aureus} [15,16]. However, only type I-V are globally distributed while others appear to exist as local strains in the country of origin [13,15]. Only the type I-V, have so far been reported in \textit{S. aureus} and type V in two isolates of \textit{S. haemolyticus} from Nigeria [14]. Several SCC\textit{mec} subtypes, subtypes IIA to E and subtypes IVa to IVg and SCC\textit{mec} type V\textsubscript{T} have been reported in the literature but no report exists from Nigeria as far as we know [13,15].

As methicillin resistance is prevalent in CoNS, methicillin-resistant CoNS (MRCoNS) may serve as a large reservoir of SCC\textit{mec} available for \textit{S. aureus} to form methicillin resistant \textit{S. aureus} (MRSA) [12,16]. Studies have shown that SCC\textit{mec} elements are more diverse in MRCoNS and new \textit{ccr} genes are still being continually identified in various strains of MRCoNS [16].

In this study, in order to examine the genetic drug resistance mechanisms in faecal isolates of CoNS, the presence of antibiotic resistance genes consisting of \textit{mecA}, \textit{erm}(\textit{A}), \textit{erm}(\textit{B}), \textit{erm}(\textit{C}), \textit{msr}(\textit{A}), \textit{tet}(\textit{M}), \textit{tet}(\textit{K}) and \textit{aac}(6\′)-\textit{aph}(2\′) and the globally distributed SCC\textit{mec} types and subtypes were analysed by PCR. This is the first report on antibiotic resistance genes of CoNS in Nigeria as well as, to our knowledge, the first report on the SCC\textit{mec} elements in human intestinal CoNS isolates.

**Methods**

**Bacterial strains**

CoNS isolates were obtained from freshly voided stool samples of apparently healthy children and adult subjects (\(n = 117\)) who came for immunizations at five healthcare institutions and households in the community of Ile-Ife, in South-Western Nigeria who gave consent for sample collection. In this study, only staphylococcal isolates were analyzed while clinical data of human subjects were not collected. The study was approved by the Obafemi Awolowo University Postgraduate Research Committee and the Review Boards of the institutions where samples were collected. Parental consent was obtained for each of the children used in the study.

**Isolation and identification of staphylococci**

All the isolates were negative to the coagulase slide and tube tests and for the \textit{S. aureus}-specific \textit{nuc} gene as determined by PCR using the protocol previously described [17]. Isolates were further identified to species level by morphological characteristics and by biochemical tests as described previously [2]. Further species differentiation was done by the Vitrek 2 apparatus (bioMerieux, Inc. Durham, NC).
Antibiotics sensitivity test
Antibiotics tested were penicillin V, oxacillin, gentamicin, erythromycin, tetracycline, co-trimoxazole, chloramphenicol, amoxicillin-clavulanate, ciprofloxacin and pefloxacin. The antibiotics screened were selected based on their use in Nigeria. Susceptibility testing was performed by the standard disc diffusion method as approved by the Clinical Laboratory Standard Institute [18].

Extraction of DNA
Genomic DNA of CoNS isolates were prepared from a 2 mL overnight Tryptone Soy Broth (Oxoid, England) culture using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich).

PCR screening of antibiotic resistance determinants
PCRs were performed on a Biometra thermocycler (Biometra, USA). All reactions were performed in a 25 μl volume containing: 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.25 mM MgCl2, 100 μM each dATP, dCTP, dGTP and dTTP; 1 μM each oligonucleotide primer, 1 U Taq polymerase (Sigma-Aldrich) and 200 ng template DNA. All strains were investigated for the presence of mecA [19]; tet(K) and tet(M) [20]; erm(A), erm(B), erm(C), msr(A) [19]; and aac(6′)-aph (2′) genes [19]. PCR products were analysed in agarose gel (1.5%) electrophoresis in 1X Tris-borate-EDTA buffer (TBE) at pH 8.3. Electrophoresis was carried out with an appropriate molecular ladder to determine fragment sizes.

SCCmec typing
SCCmec typing was performed using the PCR schemes previously published [14,15,20,21]. A single PCR was performed for each gene. For isolates in which SCCmec could not be typed, classes of the mec gene complex and the ccr gene complex (ccrAB1, ccrAB2, ccrAB3 and ccrC1) were examined by additional PCRs using the primers described previously [14]. SCCmec types were assigned based on the mec complex classes and the ccr gene types according to the criteria set for S. aureus [14,15].

Positive control strains used in the determination of the SCCmec type were the MRSA strains COL/SCCmec type I-ST250, BK2464/SCCmec type II- ST5, HUSA304/SCCmec type III- ST239, PL72/SCCmec type IV-ST5 and BK2529/SCCmec type V-ST8 [17]. As no control strains were available for the remaining SCCmec type IV subtypes, we run the simplex PCRs of each using available protocols and correlating the amplicon sizes obtained with those of the literature [15].

Results
Carriage of CoNS strains by subjects
From 117 subjects screened, 53 staphylococcal isolates were obtained; in particular S. epidermidis (n = 20), S. haemolyticus (n = 10), S. saprophyticus (n = 5), S. capitis (n = 5), S. lugdunensis (n = 2), S. warneri (n = 4), S. xylosus (n = 4), and S. cohnii (n = 3).

Antibiotics susceptibility testing
Resistance rate was generally low in all isolates showing 100.0%, 98.1%, 94.3%, 92.5%, 90.6%, and 86.8% susceptibility to pefloxacin, ciprofloxacin, gentamicin, chloramphenicol, erythromycin, and tetracycline, respectively (Table 1). Higher resistance rate were obtained for amoxicillin-clavulanic acid (58.5%) and co-trimoxazole (35.8%). All the organisms were resistant to Penicillin V. Oxacillin-resistant isolates were 28.3% of total.

PCR for the detection of antibiotic resistance genes
Correlation between phenotypes and genotypic traits of resistance to the antibiotics was absolute. The aac(6′)-aph (2′) gene was detected in all the three isolates resistant to gentamicin while four out of the five erythromycin resistant isolates (2 S. epidermidis, 2 S. haemolyticus, 1 S. cohnii) were positive to erm(C). The remaining S. haemolyticus isolate had msr(A) gene. The tet(K) gene was detected in 6 (3 S. haemolyticus, 1 S. xylosus, 1 S. capitis, 1 S. cohnii) out of the 7 tetracycline resistant isolates while 4 (2 S. haemolyticus, 1 S. xylosus and 1 S. capitis) possessed the tet(M) gene. Three of the isolates (S. haemolyticus, S. xylosus and S. capitis) had both genes. All the fifteen oxacillin resistant isolates possess the mecA gene and were taken as MRCoNS.

SCCmec typing
SCCmec types were assigned for 13 of the mecA positive isolates (Table 2). SCCmec type comprised of SCCmecI-ccrAB2-a2 (4 isolates: 3 S. epidermidis, 1 S. warneri), SCCmecIvb- ccrAB2-a3 (1 isolate: S. epidermidis), SCCmecIVd- ccrAB2-a3 (8 isolates: 3 S. epidermidis, 2

| Table 1 | Antibiotic resistance of CoNS isolates from faecal samples |
|---------|----------------------------------------------------------|
| Antimicrobial | MRCoNS (n = 15) | MSCoNS (n = 38) | Total (n = 53) |
| Penicillin V (PEN) | 15 (100) | 38 (100) | 53 (100) |
| Oxacillin (OKA) | 15 (100) | 0 (0) | 15 (28.3) |
| Gentamicin (GEN) | 1 (6.7) | 2 (5.3) | 3 (5.7) |
| Erythromycin (ERY) | 1 (6.7) | 4 (10.5) | 5 (9.4) |
| Tetracycline (TET) | 1 (6.7) | 6 (15.8) | 7 (13.2) |
| Co-trimoxazole (COT) | 14 (93.3) | 5 (13.2) | 19 (35.8) |
| Chloramphenicol (CL) | 2 (13.3) | 2 (5.3) | 4 (7.5) |
| Amoxicillin-clavulanic acid (AMC) | 15 (100) | 16 (42.1) | 31 (58.5) |
| Ciprofloxacin (CIP) | 1 (6.7) | 0 (0) | 1 (1.9) |
| Pefloxacin (PEF) | (0) | 0 (0) | 0 (0) |
| Strain (Unique strain ID) | Phenotype | Genotype |
|--------------------------|-----------|----------|
| **Phenotype**             | **nuc**   | **meca** | **aac-aph** | **erm(A)** | **erm(B)** | **erm(C)** | **msrA** | **tetM** | **tetK** | **SCCmec type** | **ccr complex** |
| **Strain (Unique strain ID)** | PEN | OXA | GEN | ERY | TET | COT | CL | CIP | nuc | mecA | aac-aph | erm(A) | erm(B) | erm(C) | msrA | tetM | tetK | |
| S. capitis (SC01)          | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. epidermidis (SE01)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | I   | ccrABβ2-a2 |
| S. epidermidis (SE02)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | I   | ccrABβ2-a2 |
| S. epidermidis (SE03)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | I   | ccrABβ2-a2 |
| S. epidermidis (SE04)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivb | ccrABβ2-a3 |
| S. epidermidis (SE05)      | R   | R   | R   | S   | S   | R   | R   | R   | -   | +   | +    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. epidermidis (SE06)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. epidermidis (SE07)      | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. epidermidis (SE08)      | R   | R   | S   | S   | S   | S   | S   | S   | -   | -   | +    | -     | -     | -     | -     | -    | +    | +   | untypable | Untypable |
| S. epidermidis (SE09)      | R   | R   | S   | S   | R   | S   | S   | S   | -   | +   | -    | -     | -     | -     | -     | +    | -    | -   | untypable | Untypable |
| S. saprophyticus (SS01)    | R   | R   | S   | S   | S   | S   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. warneri (SW01)          | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | I   | ccrABβ2-a3 |
| S. warneri (SW02)          | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. xylosus (SX01)          | R   | R   | S   | S   | S   | R   | R   | R   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. xylosus (SX02)          | R   | R   | S   | S   | S   | R   | S   | S   | -   | +   | -    | -     | -     | -     | -     | -    | -    | -   | Ivd | ccrABβ2-a3 |
| S. capitis (SC02)          | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | +    | +   | -     | -     |
| S. capitis (SC03)          | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | +    | +   | -     | -     |
| S. capitis (SC04)          | R   | S   | S   | S   | S   | R   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | +    | +   | -     | -     |
| S. capitis (SC05)          | R   | S   | R   | S   | S   | S   | S   | S   | -   | -   | +    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. cohnii (SCO01)          | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. cohnii (SCO02)          | R   | S   | S   | R   | R   | S   | R   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | +    | +   |
| S. cohnii (SCO03)          | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE10)      | R   | S   | R   | S   | S   | S   | S   | S   | -   | -   | +    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE11)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE12)      | R   | S   | S   | S   | S   | R   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE13)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE14)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE15)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE16)      | R   | S   | R   | R   | R   | R   | R   | R   | -   | -   | +    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE17)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| S. epidermidis (SE18)      | R   | S   | S   | S   | S   | S   | S   | S   | -   | -   | -    | -     | -     | -     | -     | -    | -    | -   | -     | -     |
| Species          | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial | Resistance | Serial |
|------------------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|
| S. epidermidis (SE19) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. epidermidis (SE20) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. haemolyticus (SH01) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. haemolyticus (SH02) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. haemolyticus (SH03) | R         | S      | S          | S      | S          | R      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | +          | +      |
| S. haemolyticus (SH04) | R         | S      | S          | S      | R          | R      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | +          | +      |
| S. haemolyticus (SH05) | R         | S      | S          | S      | R          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | +          | +      |
| S. haemolyticus (SH06) | R         | S      | S          | R      | R          | R      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | +          | +      |
| S. haemolyticus (SH07) | R         | S      | R          | S      | R          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | +          | +      |
| S. haemolyticus (SH08) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. haemolyticus (SH09) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. haemolyticus (SH10) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. lugdunensis (SL01) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. lugdunensis (SL02) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. saprophyticus (SS02) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. saprophyticus (SS03) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. saprophyticus (SS04) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. saprophyticus (SS05) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. warneri (SW03) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. warneri (SW04) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. xylosus (SX03) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| S. xylosus (SX04) | R         | S      | S          | S      | S          | S      | S          | S      | -          | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| Summary          | R=53       | R=15   | R=3        | R=5    | R=7        | R=19   | R=4        | R=1    | +=0        | +1.5   | +3         | +0    | +=0        | +4    | +=1        | +=4   | +=6        |

R, Resistant; S, susceptible; +, positive in specific PCR; −, negative in specific PCR.
S. xylosus, 1 S. saprophyticus, 1 S. warneri, 1 S. capitis). Two of the mecA positive isolates (S. epidermidis) were found to be untypable.

**Discussion**

Coagulase-negative staphylococci (CoNS) isolates from various sources have been identified as reservoir of genetic determinants of antibiotic resistance such as antibiotic resistance genes and various SCCmec elements [16]. The horizontal transfer of these resistance genes is thought to contribute to the reported increasing rate of resistance in strains of these organisms as well as in S. aureus. In the same vein, the horizontal transfer of SCCmec elements has been thought to contribute to the generation of new strains of methicillin resistant staphylococci, including MRSA. One of the environments which favour such an exchange is the gastrointestinal tract [22] and this study thus focused on the presence of these genes in CoNS present in the gut and isolated from stool samples of healthy individuals from Nigeria.

Different rates of resistance were recorded for the various antibiotics tested and full correlation between phenotypes and genotypic traits of resistance to the antibiotics was found. Erythromycin resistance in staphylococci has been reported to be predominantly mediated by erythromycin-resistant methylase encoded by the  erm genes [6,23], namely  erm(A),  erm(B) and  erm(C).  erm(A) is found on the transposon \( \text{Tn554} \) with a single specific site for insertion into the S. aureus chromosome while  erm(B) gene is found on the transposon \( \text{Trn551} \) of a penicillinase plasmid. The  erm(C) gene on the other hand is responsible for constitutive or inducible resistance to erythromycin and is generally located on small plasmids [5,6,23]. This indicates the high capacity of these genes to be horizontally transferred to recipient strains. Our study showed that 4 (S. epidermidis 2, S. haemolyticus 1, S. colnii 1) out of 5 erythromycin resistant isolates possessed  erm(C) genes. The  erm(A) and  erm(B) genes were absent. Studies conducted in other countries such as Italy, Denmark, and Tunisia also reported  erm(C) as the prevalent gene in clinical isolates of erythromycin resistant S. epidermidis [7,23,24]. One of the erythromycin resistant S. haemolyticus strain was found to possess the  msr (A) gene which encodes an ATP-dependent efflux pump conferring resistance to 14- and 15-membered macrolides [5]. Six of the tetracycline resistant strains (3 S. haemolyticus, 1 S. capitis, 1 S. xylosus, and one S. colnii ) were also found to possess the  tet(K) gene which encodes for an efflux mechanism of resistance. The presence of these efflux pumps in the CoNS strains from stool samples may contribute to the increase in incidence of resistance to other antimicrobial agents that are targeted by these efflux pumps, such as some antiseptics and disinfectants. The overall prevalence of tetracycline resistance is noteworthy and may reflect the overuse of different tetracyclines in the study area. Despite the fact that tetracycline is not officially recommended for children in the study area, tetracycline capsules are widely available in all stores in Nigeria and it is one of the most used drugs in this country. On the other hand, co-trimoxazole was the first line oral antibiotic recommended by World Health Organisation’s Integrated Management of Childhood Illnesses (IMCI) for the treatment of local bacterial infection in the infant and thus it is widely prescribed by many physicians and is often used as a prophylaxis in many diseases in the study area. Hence the high resistance rate obtained for it may not be out of place. The same applies to amoxicillin-clavulanate which are often prescribed instead of β-lactam susceptible penicillins in the study area.

It appears that the incidence of CoNS isolates from faeces carrying aminoglycosides, macrolides, and tetracycline resistant genes may be due to the transfer of resistance genes inter- and intra species thereby contributing to the dissemination of staphylococcal resistance. The association between methicillin resistance and resistance to antibiotics belonging to classes other than beta-lactam is of particular interest. For instance the set of MRCoNS included in this study presents some examples. Strain SEO5 is resistant to aminoglycosides and is positive to SCCmecIVd, the structure of which is known to be lacking genetic determinants responsible for resistance to aminoglycosides. Conversely SCCmec type II and IVc carry within them PUB110 and Tn4001, respectively. By comparison to other S. epidermidis within the MSCoNS subgroup, it can be concluded that the element carrying the aminoglycoside resistance gene is outside the SCCmec (see strain SE10). Of note is the isolation of strains possessing a pattern of multi-resistance (e.g. SEO5 and SX01). This finding is interesting as samples were isolated from healthy people. Multi-resistance is more often recorded in the hospital settings and in the case of staphylococci, is associated with the use of medical devices such as catheters (25). This information is important for the control of nosocomial infections and confirms the importance of CoNS as a reservoir of resistance determinants. In addition to this, given the extensive use of these antibiotics in the study area, the widespread occurrence of resistance mechanisms with potential for rapid dissemination necessitates the implementation of surveillance programmes to monitor the development and spread of antimicrobial resistance in our country.

In agreement with previous studies [16,25], the SCCmec elements identified in the MRCoNS strains investigated herein exhibited some genetic diversity. Previous reports have indicated that type VI, VII, IX, X and XI are yet to be reported in MRCoNS and type I and VIII are still rare while type II, III, IV and V were more common [11,16,25,26]. Our results are in general agreement with these reports. However, in contrast to an earlier report [25] which found SCCmecIII as the most common SCCmec element (39.3%)
followed by SCCmecV (36.9%) and SCCmecIV (20.2%), our results indicated that SCCmecIVd was the most prevalent (53.3%) followed by SCCmecI (26.7%) and SCCmecIVb (6.7%). It had been suggested that the variations in the distribution of different types of SCCmec in MRCoNS depend on the host species and on the geographical locations [25,26]. Our results indicated that most of the type IVd strains isolates were S. epidermidis whereas a study conducted in the Netherland reported a prevalence of type IVc in S. epidermidis and other staphylococci isolated from pigs [16]. Other studies have found type V SCCmec associated with S. haemolyticus [16,27]. The higher prevalence of SCCmecIV in our strains could be explained by the fact that all the samples in our study were from the community, where this SCCmec-type is diffused, whereas the type I, II and III SCCmec are more frequently isolated in hospital settings. There is also a variation in the distribution and prevalence of the various SCCmec types in MRCoNS in different countries [26]. SCCmec type III has been found to be the most prevalent in southern Brazil (52%), SCCmec type IV in the United Kingdom (36%), type IVa in Japan (40.8%), and type II in China. Some authors have recently reported type V and untypable elements in two S. haemolyticus isolates from Nigeria [27]. Our data add on to this latter study providing information for CoNS other than S. haemolyticus circulating in Nigeria.

SCCmec could not be classified in two of the MRCoNS isolates. They may belong to other SCCmec types not considered in the present investigation or may be among those that cannot be assigned to by currently-available PCR-based methods. Nevertheless the design and validation of a comprehensive SCCmec typing classification scheme for MRCoNS is heavily challenged by the frequent isolation of strains possessing “non-typeable” elements or even positive to more than one SCCmec-type [16,25]. In our study, SCCmec type were assigned by PCR protocols originally developed for SCCmec in MRSA [14,15], supporting the general conclusion that the scheme is still suitable as a first screening of SCCmec types in MRCoNS. Our results also indicate a large diversity in the J1 region in type IV of SCCmec and a large diversity and heterogenous reservoir of SCCmec among the MRCoNS isolated from faecal samples of humans. This may be a risk for interspecies horizontal transfer of new SCCmec types between CoNS and S. aureus [28]. The hypothesis of the particular case of SCCmec transfer between S. epidermidis and S. aureus has also been reported [11]. Although direct proof of transfer was not obtained in this study, SCCmec type IVd was present in 8 MRCoNS of various species indicating the possibility of interspecies transfer of SCCmec elements in CoNS strains in the gastrointestinal tracts.

**Conclusion**

In conclusion, our study indicated that CoNS colonising the gastrointestinal tracts of healthy individuals may represent a reservoir of different antibiotic resistance genes and SCCmec elements.

**Abbreviations**

CoNS: Coagulase-negative staphylococci; SCCmec: Staphylococcal chromosome cassette mec; ccr: Cassette chromosome recombinase; MRSA: Methicillin resistant S. aureus; MRCoNS: Methicillin-resistant CoNS.

**Competing interests**

The authors declared that they have no competing interest.

**Authors’ contributions**

AL, EOA and LAV conceived of the study and participated in its design. AL and LAV participated in the coordination and helped to draft the manuscript. EOA carried out the phenotypic and molecular characterization of the isolates and drafted the manuscript. LAV and DP participated in the molecular genetic studies. MP participated in the co-ordination of the study. All authors read and approved the final manuscript.

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**Author details**

1. School of Pharmacy, University of Camerino, Camerino, Italy. 2. School of Biosciences and Biotechnology, University of Camerino, Camerino, Italy. 3. Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

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**References**

1. Piette A, Verschraegen G: Role of coagulase-negative staphylococci in human disease. Vet Microbial 2009, 134(1):45–54.
2. Alinkluini E, Lamarina A: Species distribution and antibiotic resistance in coagulase-negative staphylococci colonizing the gastrointestinal tract of children in Ile-Ife, Nigeria. Trac J Pharm Res 2010, 9(1):35–43.
3. Archer GL, Climo MW: Antimicrobial susceptibility of coagulase-negative staphylococci. Antimicrob Agents Chemother 1994, 38:2321–2327.
4. Gatemann SC, Kocinski T, Friedrich S: Distribution and expression of macrolide resistance genes in coagulase-negative staphylococci. Clin Microbiol Infect 2007, 13(8):777–781.
5. Leclercq R: Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 2002, 34(4):482–492.
6. Zmantar T, Kousidi B, Miladi H, Bakhrouf A: Detection of macrolide and disinfectant resistance genes in clinical Staphylococcus aureus and coagulase-negative staphylococci. BMC Research Notes 2011, 4(1):453.
7. Ardic N, Ozurt M, Sareyupoglu B, Haznedaroğlu T: Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. Int J Antimicrob Agents 2005, 26(3):213–218.
8. Udo EE, Danhi AA: Detection of genes encoding aminoglycoside-modifying enzymes in staphylococci by polymerase chain reaction and dot blot hybridization. Int J Antimicrob Agents 2000, 15(4):275–279.
9. Hooper DC: Fluoroquinolone resistance among gram-positive cocci. Lancet Infect Dis 2002, 2:530–538.
10. Weisman LE: Coagulase-negative staphylococcal disease: emerging therapies for the neonatal and pediatric patient. Curr Opin Infect Dis 2004, 17(3):237–241.
11. Hanssen AM, Ericson Solllid Ju: SCCmec in staphylococci: genes on the move. FEMS Immunol & Med Microbial 2006, 46(1):8–20.
12. International Working Group on the Classification of Staphylococcal Cassette Chromosome E: Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 2009, 53(12):4961–4967.
Vitali et al. BMC Microbiology 2014, 14:106
http://www.biomedcentral.com/1471-2180/14/106

13. Zhang K, McClure J-A, Elayed 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(2):531–540.

14. Zhang K, McClure J-A, Elayed S, Louie T, Conly JM: Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant staphylococcus aureus. J Clin Microbiol 2005, 43(10):5026–5033.

15. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, van Belkum A, Neela V: A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome mec types in meticillin-resistant staphylococcus aureus. J Med Microbiol 2010, 59(Pt 10):1135–1139.

16. Tulinski P, Fluit AC, Wagenaar JA, Mevius D, van de Vijver L, Duim B: Methicillin-resistant coagulase-negative staphylococci on pig farms as a reservoir of heterogeneous staphylococcal cassette chromosome mec elements. Appl Environ Microbiol 2012, 78(2):299–304.

17. Petrelli D, Repetto A, D’Ecole S, Ramboni S, Ripa S, Preyna M, Vitali LA: Analysis of meticillin-susceptible and meticillin-resistant biofilm-forming Staphylococcus aureus from catheter infections isolated in a large Italian hospital. J Med Microbiol 2008, 57(3):364–372.

18. CLSI: Performance standards for antimicrobial susceptibility testing, 20th informational supplement. M100–S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. Clinical and Laboratory Standards Institute.

19. Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouquelle M, Bergeron MG: Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of staphylococcus aureus and staphylococcus epidermidis. Antimicrob Agents Chemother 2000, 44(2):231–238.

20. Ng LK, Martin I, Alfa M, Mulvey M: Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 2001, 15(4):209–215.

21. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O’Brien FG, Coombs GW, Pearson JW, Tenover FC, Kapi M: Dissemination of new methicillin-resistant staphylococcus aureus clones in the community. J Clin Microbiol 2002, 40(11):4289–4294.

22. de Vries LE, Vallaës Y, Agena Y, Vaishampayan PA, Garcia-Montaner A, Kuehl JV, Chrestensen H, Barlow M, Francino NP: The gut as reservoir of antibiotic resistance: microbial diversity of tetracycline resistance in mother and infant. PLoS One 2011, 6(6):e1644.

23. Westh H, Hougaard DM, Vuust J, Rosdahl VT: erm genes in erythromycin-resistant staphylococcus aureus and coagulase-negative staphylococci. APMIS 1995, 103(3):225–232.

24. Petrelli D, Zampaloni C, D’Ecole S, Preyna M, Ballarin P, Ripa S, Vitali LA: Analysis of different genetic traits and their association with biofilm formation in staphylococcus epidermidis isolates from central venous catheter infections. Eur J Clin Microbiol Infect Dis 2006, 25(12):773–781.

25. Zong Z, Peng C, Lu X: Diversity of SCCmec elements in meticillin-resistant coagulase-negative staphylococci clinical isolates. PLoS One 2011, 6(5):e19171.

26. Ruppe E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, Benchouk S, Hassaine H, Maiga I, Dallal M: Diversity of staphylococcal cassette chromosome mec structures in meticillin-resistant staphylococcus epidermidis and staphylococcus haemolyticus strains among outpatients from four countries. Antimicrob Agents and Chemother 2009, 53(2):442–449.

27. Shittu A, Oyedara O, Abegunrin F, Okon K, Raji A, Taiwo S, Ogunsola F, Onyedibe K, Blisha G: Characterization of meticillin-susceptible and resistant staphylococci in the clinical setting: a multicentre study in Nigeria. BMC Infect Dis 2012, 12(1):286.

28. Wielders CLC, Vriens MR, Brisse S, de Graaf-Miltngen LAM, Troelstra A, Fleer A, Schmitz VR, Verhoef J, Fluit AC: Evidence for in-vivo transfer of mecA DNA between strains of staphylococcus aureus. Lancet 2001, 357(9269):1674–1675.

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