

Research Paper

Methicillin-resistant coagulase-negative staphylococci from healthy dogs in Nsukka, Nigeria

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Abstract

The occurrence, resistance phenotype and molecular mechanisms of resistance of methicillin-resistant staphylococci from groin swabs of 109 clinically healthy dogs in Nsukka, Nigeria were investigated. The groin swab samples were cultured on mannitol salt agar supplemented with 10 µg of cloxacillin. Sixteen methicillin-resistant coagulase negative staphylococci (MRCoNS), all harbouring the *mecA* gene were isolated from 14 (12.8%) of the 109 dogs studied. The MRCoNS isolated were: *S. sciuri* subspecies *rodentium*, *S. lentus*, *S. haemolyticus*, and *S. simulans* with *S. sciuri* subspecies *rodentium* (62.5%) being the predominant species. Thirteen (81.3%) of the MRCoNS were resistant to tetracycline while 12 (75%) and 10 (62.5%) were resistant to kanamycin and trimethoprim-sulphamethoxazole respectively. None of the isolates was resistant to fusidic acid, linezolid and vancomycin. Thirteen (81.3%) of the MRCoNS were multi-drug resistance (MDR). Other antimicrobial genes detected were: *blaZ*, *tet(K)*, *tet(M)*, *tet(L)*, *erm(B)*, *lmu(A)*, *aacA-aphD*, *aphA3*, *str*, *dfr(G)*, *cat_{pC221}*, and *cat_{pC223}*. Methicillin-resistant staphylococci are common colonizers of healthy dogs in Nigeria with a major species detected being *S. sciuri* subsp. *rodentium*.

Key words: methicillin-resistant, *S. sciuri*, resistant genes, dogs.

Introduction

Staphylococci are Gram-positive cocci usually found as transient normal flora of the skin and mucous membranes of mammals and birds and can easily spread to humans by contact and through formites (Fusi Ngwa *et al.*, 2007). According to Bergeron *et al.* (2011) the *Staphylococcus* genus groups together 45 species and 21 subspecies which are classified in two groups based on their ability to produce the enzyme coagulase: coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS).

Thirty-eight different species are found within the CoNS group, including the *S. sciuri* subgroup, which consists of 3 species and 3 subspecies (*S. sciuri* subspecies *rodentium*, *S. sciuri* subspecies *sciuri*, *S. sciuri* subspecies *carnaticus*; *S. lentus*, and *S. vitulinus*) (Kloos and Ban-

nerman, 1994). *Staphylococcus sciuri*, first described in 1976 by Kloos *et al.* (1976), is the most abundant member of the genus *Staphylococcus* with a wide habitat range (Kloos and Bannerman, 1994; Kloos *et al.*, 1997). Although CoNS are saprophytic and rarely pathogenic (Kloos *et al.*, 1997), multi-drug resistant strains have been associated with severe cases of difficult to treat infections, especially in immune compromised individuals (Zell *et al.*, 2008).

Companion animals, particularly dogs and cats, are frequently implicated as potential reservoirs of methicillin-resistant staphylococci (Saleha and Zunita, 2010). Prevalence of staphylococcal species in companion animals is important because of the potential for zoonotic infections and possibility of resistance genes transfer (Rachal *et al.*, 2009). Human colonization and infections with

methicillin-resistant staphylococci (MRS) have been reported in several parts of Nigeria (Fusi Ngwa *et al.*, 2007; Olowe *et al.*, 2007; Ghebremedhin *et al.*, 2009). However, no information exists on the occurrence and molecular characteristics of MRS from dogs in Nigeria. Thus, the objective of this study was to determine the occurrence, resistance phenotypes and molecular mechanisms of resistance of MRS in healthy dogs in Nsukka, Nigeria.

Material and Methods

Sample collection and processing

Dogs from the university town of Nsukka and four other surrounding communities (Ibagwa Aka, Obollo Afor, Orba, and Opi) were used for the study. A total of 109 clinically healthy dogs consisted of 20 individual household-dogs from the university town, 50 dogs displayed for sale in the local markets in the four communities, and 39 dogs presented to the University of Nigeria Veterinary Teaching Hospital, Nsukka for routine vaccination were sampled. Informed consent of the dog owners was obtained prior to sample collection. The cotton tip of each swab stick was moistened in sterile normal saline and gently rolled over the groin area for about 10 seconds. Each swab sample was streaked on mannitol salt agar (MSA; Oxoid, Basingstoke) supplemented with 10 µg/mL of cloxacillin. Inoculated plates were incubated at 37 °C for 24 to 48 h. On each plate that produced growth, three colonies were randomly selected and purified on nutrient agar. Purified colonies were subjected to Gram staining and catalase test and presumptive staphylococcal colonies (Gram-positive cocci in bunches with positive catalase test) were further evaluated for coagulase production (tube coagulase using rabbit plasma) and haemolysis on 5% sheep blood agar using standard procedures. Representative colony/colonies (if different morphology was shown) from each sample was/were selected for further analysis, including molecular identification.

Identification of staphylococci and methicillin-resistant staphylococci

Coagulase-negative staphylococci were identified by PCR amplification followed by sequencing of *sodA* gene (Poyart *et al.*, 2001). The presence of *mecA* in all recovered isolates was investigated by PCR (Gómez-Sanz *et al.*, 2011).

Antimicrobial resistance pheno- and genotype

Antimicrobial susceptibility of the methicillin-resistant staphylococcal isolates to 17 antimicrobial agents was determined by the agar disk-diffusion method. Antimicrobial agents tested were as follows: penicillin (10 U), oxacillin (1 µg), cefoxitin (30 µg) erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), kana-

mycin (30 µg), streptomycin (10 U), tobramycin (10 µg), tetracycline (30 µg), trimethoprim-sulphamethoxazole (25 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), mupirocin (200 µg), fusidic acid (10 µg), vancomycin (30 µg), and linezolid (30 µg). The disk-diffusion method and breakpoints recommended by Clinical and Laboratory Standards Institute (2010) were employed for all antimicrobials except for streptomycin and fusidic acid, where the methods and breakpoints recommended by the Société Française de Microbiologie (<http://www.sfm.asso.fr>) were used. The double-disk diffusion test (D-test) was performed on all isolates to detect inducible clindamycin resistance.

The presence of the following 30 antimicrobial resistance genes was investigated by PCR: *mecA*, *blaZ*, *tet(K)*, *tet(M)*, *tet(L)*, *erm(A)*, *erm(B)*, *erm(C)*, *erm(T)*, *mph(C)*, *msr(A)*, *msr(B)*, *lnu(A)*, *vga(A)*, *vga(C)*, *aacA-aphD*, *aphA-3*, *aadD*, *aadE*, *aadA*, *str*, *dfr(A)*, *dfr(D)*, *dfr(G)*, *dfr(K)*, *fexA*, *cfr*, *cat_{PC194}*, *cat_{PC221}*, and *cat_{PC223}* (Schwarz *et al.*, 2001; Hanselman *et al.*, 2008). Positive controls from the collection of the University of La Rioja were included in each PCR reaction.

Results

Occurrence of methicillin-resistant staphylococcal species

Sixteen methicillin-resistant coagulase negative staphylococci (MRCoNS), all harbouring the *mecA* gene were isolated from 14 (12.8%) of the 109 dogs sampled. The MRCoNS isolated belonged to four different species namely: *S. sciuri* subspecies *rodentium*, *S. lentus*, *S. haemolyticus*, and *S. simulans*, with *S. sciuri* subspecies *rodentium* (62.5%) being the predominant species detected (Figure 1).

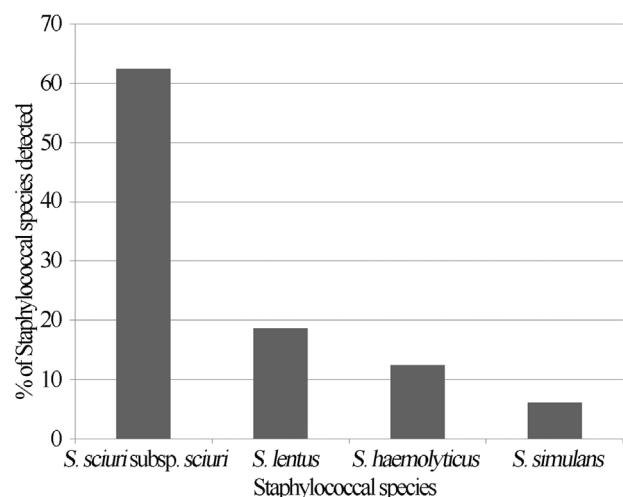


Figure 1 - Occurrence (%) of MRCoNS species in healthy dogs in Nsukka, Nigeria.

Resistance pheno- and genotype of the MRS to other antimicrobials

Resistance phenotype of the staphylococcal isolates investigated as well as their corresponding molecular mechanisms of resistance are shown in Table 1. Thirteen (81.3%) of the MRCoNS were resistant to tetracycline while 12 (75%) and 10 (62.5%) were resistant to kanamycin and trimethoprim-sulphamethoxazole respectively. None of the isolates was resistant to fusidic acid, linezolid and vancomycin. High rate of multi-drug resistance (MDR) was observed among the MRCoNS as 13 (81.3%) of them were resistant to more than 3 classes of antimicrobial agents.

The percentage of each antimicrobial resistance gene and/or gene combination detected among the MRCoNS isolates is presented in Figure 2. The nine (56.3%) erythromycin resistant isolates were also resistant to clindamycin. Constitutive clindamycin resistance was observed in all but 3 MRCoNS isolates. These 3 isolates revealed the typical D-shaped halo around clindamycin disk, characteristic of inducible clindamycin resistance. Table 1 and Figure 2 show the different resistance genes detected among isolates. In some cases, the resistance mechanism implicated could not be detected even though a wide variety of resistance genes were tested. Six of the 10 MRCoNS that were also resistant to trimethoprim-sulphamethoxazole lacked any of the so far described genes encoding trimethoprim resistance [*dfr*(A), *dfr*(D), *dfr*(G), *dfr*(K)]. Based on these results, trimethoprim (5 µg/mL) and sulfonamide (20 µg/mL) antimicrobial agents were tested separately (CLSI 2010) and the results revealed that all trimethoprim-sulphamethoxazole resistant strains were also trimethoprim resistant. One methicillin-resistant *S. sciuri* subspecies *rodentium* (C2867) was resistant to mupirocin but *mupA* gene was not detected.

Discussion

Resistance of staphylococci to methicillin and other antimicrobials is a global problem in the chemotherapy of staphylococcal infections. As pointed out by Huebner and Goldmann (1999), this resistance has underscored the need for species identification which is an important step for monitoring the reservoir and distribution of these bacteria. In the present study, the major MR staphylococcal group present in the groin area of dogs in Nsukka was CoNS, with *S. sciuri* subspecies *rodentium*, being the predominant species and subspecies identified. Although CoNS are saprophytic and rarely pathogenic (Kloos and Bannerman, 1994), they have been associated with opportunistic infections, especially in immunocompromised individuals (Zell *et al.*, 2008). Previous studies have shown *S. sciuri* to be present not only as part of the skin, nasal and oral microflora of healthy dogs, but also as a causative agent of infections, although at lower rates than *S. pseudintermedius*

Table 1 - Species, antimicrobial resistance phenotype and resistance genes detected among MRCoNS isolated from dogs in Nsukka, Nigeria.

Strain ID	Staphylococcal species	Resistance phenotype ^a	Resistance genes detected
C2854	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN-TOB-SXT-CHL	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>aacA-aphD</i> , <i>cat</i> _{pC223}
C2869	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN-SXT-CHL-CIP	<i>mecA</i> , <i>tet</i> (K), <i>erm</i> (B), <i>lnu</i> (A), <i>aacA-aphD</i> , <i>cat</i> _{pC221}
C2853	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN-STR-SXT	<i>mecA</i> , <i>tet</i> (K), <i>erm</i> (B), <i>aacA-aphD</i>
C2865	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN-STR-SXT	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>lnu</i> (A), <i>aacA-aphD</i>
C2855	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN-SXT-CHL	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>lnu</i> (A), <i>aacA-aphD</i> , <i>cat</i> _{pC221}
C2867	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-KAN-SXT-MUP	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>lnu</i> (A), <i>aacA-aphD</i>
C2864	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>aacA-aphD</i>
C2866	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>erm</i> (B), <i>lnu</i> (A), <i>aacA-aphD</i>
C2852	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX-TET-ERY-CLI-GEN-KAN	<i>mecA</i> , <i>cat</i> _{pC223}
C2871	<i>S. sciuri</i> subsp. <i>Rodentium</i>	PEN-OXA-FOX	<i>mecA</i>
C2861	<i>S. lentus</i>	PEN-OXA-FOX-TET-ERY-CLI-KAN-SXT-CHL	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>tet</i> (L), <i>erm</i> (B), <i>aacA-aphD</i> , <i>dfr</i> (G), <i>cat</i> _{pC221}
C2860	<i>S. lentus</i>	PEN-OXA-FOX-TET-STR-SXT	<i>mecA</i> , <i>str</i> , <i>dfr</i> (G)
C2871-2	<i>S. lentus</i>	PEN-OXA-FOX-TET-KAN	<i>mecA</i> , <i>tet</i> (K), <i>tet</i> (M), <i>aacA-aphD</i>
C2873	<i>S. haemolyticus</i>	PEN-OXA-FOX-TET-KAN-SXT-CHL	<i>mecA</i> , <i>tet</i> (K), <i>aacA-aphD</i> , <i>aphA3</i> , <i>dfr</i> (G), <i>cat</i> _{pC221}
C2870	<i>S. haemolyticus</i>	PEN-OXA-FOX-TET-KAN-SXT-CHL	<i>bla</i> _Z , <i>mecA</i> , <i>tet</i> (K), <i>aacA-aphD</i> , <i>dfr</i> (G), <i>cat</i> _{pC221}
C2857	<i>S. simulans</i>	PEN-OXA-FOX	<i>mecA</i>

^aPEN, penicillin; OXA, oxacillin; FOX, cefoxitin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; KAN, kanamycin; TOB, tobramycin; STR, streptomycin; SXT, trimethoprim-sulphamethoxazole; CHL, chloramphenicol; MUP, mupirocin; CIP, ciprofloxacin.

^bMRCoNS, Methicillin-resistant coagulase negative staphylococci.

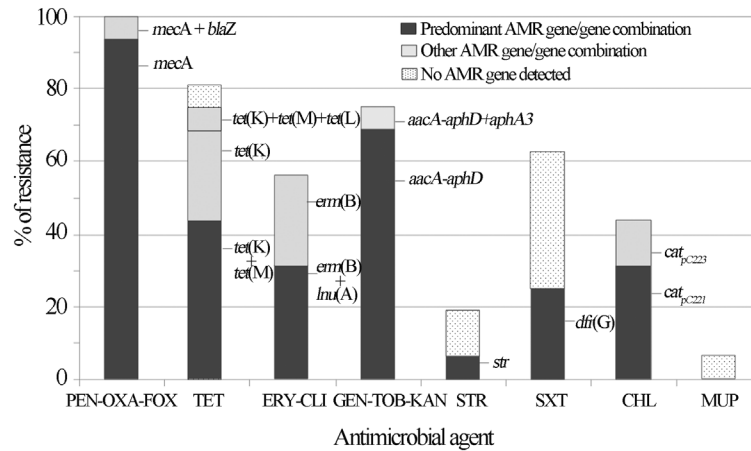


Figure 2 - Frequencies (%) of antimicrobial resistance (AMR) detected among the MRCoNS investigated in this study and resistance gene or gene combinations harboured by the resistant staphylococci. PEN, penicillin; OXA, oxacillin; FOX, cefoxitin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; KAN, kanamycin; TOB, tobramycin; STR, streptomycin; SXT, trimethoprim-sulphamethoxazole; CHL, chloramphenicol; MUP, mupirocin.

(Stepanovic *et al.*, 2001; Hauschild and Wójcik, 2007). *Staphylococcus sciuri* was among the staphylococcal strains isolated from cases of canine pyoderma in Natal City, Brazil (Lima *et al.*, 2012). A case of human wound infection by a multidrug resistant strain of *S. sciuri* has been reported (Coimbra *et al.*, 2011). Thus, this staphylococcal species could constitute a health risk to both humans and animals especially when the natural mucocutaneous barrier is breached. *Staphylococcus sciuri* has been described as a natural reservoir of the methicillin resistance gene, *mecA* (Kloos *et al.*, 1997; Couto *et al.*, 2000), which may explain the high rate of *mecA* positive *S. sciuri* recorded in the present study. The other MRCoNS isolated in this study, although at low rates, have also been reported in dogs as commensal and/or pathogens (Hauschild and Wójcik, 2007).

The absence of MRCoPS colonization among the tested dog population is similar to the findings of Vengust *et al.* (2006) and Baptiste *et al.* (2005) who failed to detect the organism in healthy dogs in Slovenian and a community in United Kingdom, respectively. However, low MRCoPS carriage rates in healthy dogs have been reported by other authors (Hanselman *et al.*, 2008; Loeffler *et al.*, 2011), with *S. pseudintermedius* as the prominent MRCoPS species, even though their occurrence is still low (0-4.5%) (Gómez-Sanz *et al.*, 2011; van Duijkeren *et al.*, 2011). Failure to detect methicillin-resistant *S. aureus* and *S. pseudintermedius* among the sampled dogs suggests that these species are not predominant methicillin-resistant staphylococci in the study area. Alternatively, groin swabs from healthy dogs were used in the present study whereas most reports on *S. pseudintermedius* in dogs used nasal, skin, perineal or combined body-site samples (Rubin and Chirino-Trejo, 2011; van Duijkeren *et al.*, 2011). It could therefore appear that the groin area of dogs is less colonized

by methicillin-resistant *S. pseudintermedius* than the mucous or skin.

High rate of MDR was observed among the MRCoNS as 81.3% of them were resistant to more than 3 classes of antimicrobial agents. Cross resistance to other antimicrobials is most common in methicillin-resistant than in methicillin-sensitive staphylococcal isolates (Orrett and Land, 2006; John and Harvin, 2007). In our MRCoNS isolates, cross-resistance was due in most cases to tetracyclines, aminoglycosides, trimethoprim-sulphamethoxazole and/or macrolides-lincosamides. Interestingly, our isolates were recovered from healthy animals whereas nosocomial strains are more likely to be MDR than commensal (John and Harvin, 2007).

Only 2 of the 16 MRCoNS (both being *S. sciuri* subspecies *rodentium*) had the same resistance phenotype; indicating a high diversity of antimicrobial resistance profiles among the strains. Interestingly, no tetracycline resistance gene was detected in 1 tetracycline-resistant MR *S. sciuri* subspecies *rodentium* (C2860) (Table 1, Figure 2). Furthermore, 2 streptomycin-resistant MR *S. sciuri* subspecies *rodentium* did not present any streptomycin resistance genes, while 5 trimethoprim-sulphamethoxazole resistant strains (again MR *S. sciuri* subsps. *rodentium*) equally did not harbour any of the so far described trimethoprim-sulphamethoxazole resistant genes for staphylococci (Table 1, Figure 2). Chromosomal mutations conferring high-level resistance to streptomycin and over-expression of the enzyme dihydrofolate reductase (DHFR), target of trimethoprim, have been described (Schwarz and Chaslus-Dancla, 2001). It is also interesting to remark that one MR *S. sciuri* subsps. *rodentium* (C2867) was resistant to mupirocin but *mupA* gene could not be detected. Previous studies have shown an association between mupirocin usage and emergence of mupirocin resistance in staphylococci (Orrett 2008). However, mupirocin is not known to be used

in veterinary practice in Nigeria, thus, mupirocin resistance in one of the *Staphylococcus* strain in the present study may not be attributed to mupirocin usage. These data altogether suggest the potential presence of novel characteristics and presents *S. sciuri* as a potential reservoir of novel antimicrobial resistant properties.

The findings in this study highlight the existence of MDR strains of MRCoNS, particularly *S. sciuri* subspecies *rodentium*, in healthy dogs in Nsukka, Nigeria. Since dogs are in close contact with their owners, the risk of transmission of MDR staphylococci between animals and humans as well as the possibility of transfer of *mecA* and other resistance genes from the CoNS to human pathogenic *S. aureus* are serious public health concerns.

Acknowledgments

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