

Antibiotic susceptibility pattern and biofilm formation in coagulase negative staphylococci

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Introduction

Coagulase negative staphylococci (CoNS) are commensals of non-sterile sites in humans and become pathogenic mostly when the host is immunocompromised by prior diseases or invasive surgical or related procedures [1]. Slime or biofilm production by CoNS has been identified as an important factor in the pathogenesis of infections as bacteria organized in biofilms are protected from the action of antibiotics and the immune system [2]. Biofilm is ascribed the most important virulence factor of *S. epidermidis* as it enables attachment and persistence of the bacteria on foreign materials [3,4]. Other studies have indicated a correlation between antibiotic resistance and slime expression. For instance, insertion of a certain transposon influences both biofilm formation and the expression of methicillin resistance in *S. epidermidis* [4]. In another study methicillin resistance was found to be significantly higher in slime positive isolates (81%) than in slime negative isolates (57%) [5]. Due to the frequent recovery of CoNS in clinical infections their antibiotic susceptibility profile as well as their biofilm forming ability was investigated in this study.

The Study

One hundred clinical strains of CoNS comprising *S. cohnii* (n = 7), *S. cohnii* ssp. *urealyticus* (n = 12), *S. epidermidis* (n = 5), *S. haemolyticus* (n = 20), *S. hominis* (n = 26), *S. intermedius* (n = 4), *S. klosii* (n = 4), *S. lugdunensis* (n = 4), *S. saprophyticus* (n = 7), *S. sciuri* (n = 5), *S. warneri* (n = 3) and *S. xylosum* (n = 3)

were isolated from various clinical specimens obtained from wards and outpatient departments of 8 medical centres from the states of Lagos and Ogun in Nigeria. The isolates were identified by cultural and biochemical characteristics. Identification to species level was performed with the VITEK-2 automated system (bioMérieux, Marcy l'Etoile, France). Susceptibility to 19 different antimicrobial agents (benzylpenicillin, oxacillin [methicillin], gentamicin, tobramycin, levofloxacin, moxifloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, tigecycline, fosfomycin, nitrofurantoin, fusidic acid, mupirocin, rifampicin and trimethoprim-sulfamethoxazole) was tested with the VITEK-2 automated system. The biofilm-forming capacity of one *S. lugdunensis* strain and five *S. epidermidis* strains obtained in this study was investigated by a method previously described [6].

Antibiotic resistance by the CoNS was highest against tetracycline (74.7%) followed by penicillin (69.5%) and fosfomycin (68.4%). Mupirocin and nitrofurantoin produced the highest *in vitro* activities against the CoNS. *Staphylococcus intermedius* and *S. xylosum* exhibited extreme resistance to most of the antibiotics tested. Resistance to methicillin was 41% (Table 1). The ratio of methicillin susceptible (MS) CoNS to methicillin resistant (MR) CoNS was approximately 2:1 (59% to 41%). High level resistance to methicillin was evident in *S. intermedius* (100%), *S. xylosum* (100%) *S. haemolyticus* (95%) and *S. epidermidis* (60%).

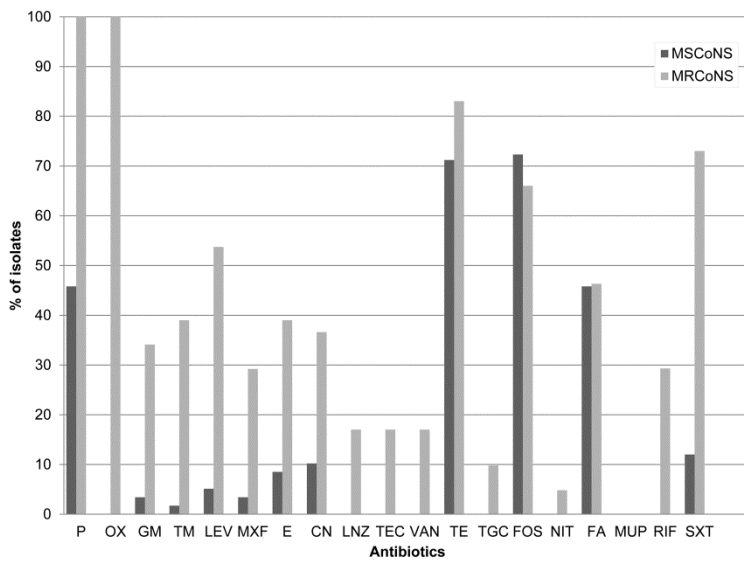
Table 1. Antimicrobial resistance profile of coagulase negative staphylococcal strains

Antibiotics	<i>S. cohnii</i>	<i>S. cohnii urealyticus</i>	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. intermedius</i>	<i>S. kloosii</i>	<i>S. lugdunensis</i>	<i>S. saprophiticus</i>	<i>S. sciuri</i>	<i>S. warneri</i>	<i>S. xylosus</i>
P	42.9	75	100	100	23.1	100	75	0	100	100	100	100
OX	28.6	25	60	95	11.5	100	0	0	42.8	0	33.3	100
GM	0	0	40	30	7.7	100	0	0	0	20	0	33.3
TM	0	0	40	25	7.7	100	0	0	0	20	0	100
LEV	0	0	40	70	7.7	100	0	0	0	20	33.3	33.3
MXF	0	0	0	25	0	100	0	0	0	20	33.3	100
E	0	16.7	40	20	7.7	100	75	0	0	0	33.3	100
CN	0	18.3	40	20	3.8	100	25	0	0	80	33.3	100
LNZ	0	0	0	0	0	100	0	0	0	0	0	100
TEC	0	0	0	0	0	100	0	0	0	0	0	100
VAN	0	0	0	0	0	100	0	0	0	0	0	100
TE	57.1	58.3	100	85	80.8	100	24	100	71.4	60	66.7	100
TGC	0	0	0	0	0	100	0	0	0	0	0	0
FOS	71.4	25	0	85	88.5	100	100	100	71.4	0	100	0
NIT	0	0	0	0	0	50	0	0	0	0	0	0
FA	100	91.7	0	10	11.5	100	75	0	100	100	33.3	100
MUP	0	0	0	0	0	0	0	0	0	0	0	0
RIF	0	0	0	10	3.8	100	0	0	14.2	0	0	100
SXT	0	0	40	90	15.4	100	25	0	57.1	0	33.3	100

*Values represent percentages

P: benzylpenicillin; OX: oxacillin; GM: gentamicin; TM: tobramycin; LEV: levofloxacin; MXF: moxifloxacin; E: erythromycin; CN: clindamycin; LNZ: linezolid; TEC: teicoplanin; VAN: vancomycin; TE: tetracycline; TGC: tigecycline; FOS: fosfomicin; NIT: nitrofurantoin; FA: fusidic acid; MUP: mupirocin; RIF: rifampicin; SXT: trimethoprim-sulfamethoxazole.

Figure 1. Antimicrobial resistance patterns of MRCoNS and MSCoNS



P: benzylpenicillin; OX: oxacillin; GM: gentamicin; TM: tobramycin; LEV: levofloxacin; MXF: moxifloxacin; E: erythromycin; CN: clindamycin; LNZ: linezolid; TEC: teicoplanin; VAN: vancomycin; TE: tetracycline; TGC: tigecycline; FOS: fosfomicin; NIT: nitrofurantoin; FA: fusidic acid; MUP: mupirocin; RIF: Rifampicin; SXT: trimethoprim-sulfamethoxazole

A comparison of the antibiotic susceptibilities of (MS) CoNS and (MR) CoNS showed that MR CoNS were more resistant (Figure 1). Biofilm formation was observed in three of the *S. epidermidis* strains tested. The *S. lugdunensis* strain did not form biofilm.

The results of the antibiotic susceptibility test showed that the β -lactam antibiotics were less effective, particularly the penicillins against CoNS. The emergence of large clusters of methicillin resistant CoNS in this study portend further therapeutic dilemma. These strains have been shown to spread within and between hospitals [7,8]. The 41% of CoNS which were methicillin resistant in this present study is encouragingly lower than the proportion (60-70%) seen in some hospitals in Europe [9]. Mupirocin's excellent activity against the CoNS is important as it will continue to be the drug of choice for decontaminating the nasal cavity of carriers of staphylococci [10].

The resistance pattern in *S. intermedius* and *S. xylosus* as seen in this present study is of major concern. Of particular interest is the 100% resistance of these two species to antibiotics (methicillin, linezolid, teicoplanin, vancomycin, tigecycline with the exception of mupirocin) that produced 100% activity against other species. The present study showed that organisms that lacked the ability to form biofilms were more susceptible to antibiotics. It is important to observe also that two of the five strains of *S. epidermidis* that failed to form biofilms represented the antibiotic sensitive clone. Our finding supports an earlier work that methicillin resistance in CoNS was higher in species that produce slime [5]. It is worthwhile to note that several of the isolates in this study could be contaminants and not real pathogens.

In conclusion, further studies are required to determine the epidemiological implication of diversified resistance in coagulase negative staphylococci.

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