

Antimicrobial resistance profiles of methicillin resistant coagulase negative *Staphylococcus* at a reference laboratory in Sierra Leone: implications for infection control

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ABSTRACT

Methicillin-resistant CoNS (MR-CoNS) are increasingly recognized as significant nosocomial pathogens. Sierra Leone lacks data on the prevalence and antibiotic-resistance patterns of these bacteria, which hinders a cross-sectoral approach to tackling antimicrobial resistance as well as regional and global health surveillance. We report on clinical multidrug-resistant MR-CoNS from Freetown, Sierra Leone, West Africa, as emerging pathogens. This study aimed to explore the prevalence and antimicrobial resistance profiles of MR-CoNS isolated from clinical samples in Freetown, Sierra Leone. A cross-sectional study was conducted at the reference laboratory from January 2025 to June 2025. Clinical samples submitted to the microbiology department were screened for *Staphylococcus* species, and isolates identified as coagulase-negative *Staphylococci* (CoNS) using standard microbiological techniques. Methicillin resistance in all isolates was tested with a 30 µg cefoxitin disc and further confirmed through an automated Scenker XK Microbial ID and AST system by measuring the minimum inhibitory concentration (MIC) with oxacillin. Antibiotic susceptibility profiles were determined using the Scenker XK Microbial ID/AST system following the Clinical and Laboratory Standards Institute (CLSI) guidelines, and data were analysed using SPSS ver 16. Findings from our study show a prevalence of 18.2% of MR-CoNS with *Staphylococcus schleiferi*, (26.9%) predominant. Linezolid, vancomycin, and teicoplanin exhibited 100% activity against all the MR-CoNS isolated. However, there was co-and multidrug resistance exhibited to commonly known antibiotics gentamycin (75-100%), levofloxacin (80-100%), clarithromycin (87-100%), including resistance to newer antibiotics as daptomycin (33-50%).

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1. INTRODUCTION

Over the past decade, coagulase-negative staphylococci (CoNS) have gained recognition as significant pathogens, with their pathogenic potential and role in various diseases increasingly

highlighted [1]. Although CoNS are part of the normal flora of human and animal skin and mucous membranes, they act as opportunistic pathogens, frequently causing infections in immunocompromised individuals [2]. These infections include skin and soft tissue infections [3], [4], bacteremia [5], ocular infections [6], urinary tract infections [7], [8], and implant-associated hospital-acquired infections.

The growing resistance of methicillin-resistant CoNS (MRCoNS) to multiple antibiotics complicates treatment strategies and underscores the need for continuous surveillance and monitoring of antibiotic susceptibility patterns [9], [10]. The rise in antibiotic-resistant CoNS in both human and non-human environments has been linked to selective pressure from healthcare practices, widespread antibiotic use in animal husbandry, and environmental exposure. Resistance to β -lactam antibiotics—particularly methicillin—poses a major concern, as it confers resistance to nearly all drugs in this class, except for two anti-methicillin-resistant *Staphylococcus aureus* (MRSA) cephalosporins [11]. This resistance is mediated by an alternative penicillin-binding protein (PBP2a) with reduced affinity for β -lactams, encoded by the *mec* genes (*mecA*, *mecB*, and *mecC*) [12]–[14]. These genes are carried on the staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element (MGE) ranging from 20 to 70 kb in size, found in staphylococci.

In 2019, Sierra Leone recorded an estimated 9,700 deaths attributable to antimicrobial resistance (AMR). Among 19 countries in the African region, it ranked fifth in age-standardized AMR-related mortality, with deaths exceeding those caused by cardiovascular diseases (9,000), maternal and neonatal disorders (8,000), and neoplasms or other non-communicable diseases (7,500) [15]. A nationwide point prevalence survey (PPS) conducted in 2021 revealed extensive antibiotic use, with 73.7% of hospitalized patients across 26 facilities receiving antibiotics, the highest prevalence observed in pediatric wards [16]. Currently, Connaught Hospital—primarily serving adult medical and surgical patients—is the only institution in Sierra Leone with an established antimicrobial stewardship (AMS) program [17]. The lack of sufficient bacteriology capacity, limited AMS initiatives, and widespread antibiotic use in public hospitals are expected to exacerbate the country's AMR burden. Methicillin-resistant *Staphylococcus* (MRS) surveillance data are sparse. Importantly, data on methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) are nonexistent, and yet these have been reported as significant nosocomial pathogens. Identifying the local prevalence and determining susceptibility patterns of these MR-CoNS is essential for addressing local antimicrobial knowledge gaps and providing data that can aid in developing targeted antimicrobial stewardship programs as well as instituting infection prevention and control strategies.

2. METHOD

This laboratory-based cross-sectional study was carried out at the Premium Medical Services Microbiology Laboratory, a facility that delivers essential diagnostic support to a diverse patient population and serves as a critical interface between healthcare-associated and community-acquired infections. The regional setting is characterized by notable variations in antimicrobial resistance, particularly methicillin-resistant coagulase-negative staphylococci (MRCoNS), driven by extensive antimicrobial use. As a central laboratory hub with high patient volume and widespread reliance on antimicrobial therapies, Premium provides an optimal environment for evaluating the prevalence and resistance patterns of MRCoNS.

2.1. Study population

The study included all patients' clinical samples sent to the microbiology department at Premium Medical Services Laboratory for microbial analyses. These samples were from consenting patients from different medical units, encompassing all age groups and sexes.

2.2. Sampling and sample size

We used a non-probability purposive sampling technique to focus only on the information-rich cases for the purposes of this study. The research, spanning a 6-month duration, included 143 distinct clinical samples.

2.3. Bacterial identification and antimicrobial susceptibility testing

Pure bacterial colonies were selected from well-isolated areas on culture plates and emulsified in the sample diluent (common) to prepare a suspension matching the 0.5 McFarland standard. This standardization ensured the correct inoculum density for accurate identification and susceptibility testing. The prepared suspension was inoculated into specialized micro-well panels (ID/AST cards) compatible with the Scenker XK-Microbial identification system. The *Staphylococcus* ID/ASTcard, with each card containing a series of wells embedded with dried biochemical substrates for identification and predefined concentrations of antibiotics for susceptibility testing, was used [18].

After inoculation, the kits were incubated for 18-24 hours, after which they were loaded into the XK-11 instrument chamber, and the test was initiated through the system's software interface. The instrument automatically incubated the cards and monitored the reactions using optical sensors. Bacterial identification was based on the colorimetric changes that occurred as the organisms metabolized specific substrates. These reactions generated a metabolic profile that was interpreted by the system's software and compared against a built-in database of known bacterial species. Simultaneously, the antimicrobial susceptibility testing was carried out by measuring bacterial growth (via turbidity changes) in the presence of different antibiotics, with the system determining minimum inhibitory concentrations (MICs) for oxacillin (OXA), gentamicin (GEN), levofloxacin (LEV), erythromycin (ERY), clindamycin (CLI), linezolid (LNZ), daptomycin (DAP), teicoplanin (TEC), vancomycin (VAN), tetracycline (TET), tigecycline (TIG), fosfomycin (FOS), fusidic acid (FUS), rifampicin (RIF), trimethoprim/sulfamethoxazole (TMP-SMX), Moxifloxacin (MOX), Penicillin (PEN), Nitrofurantoin, Tigecycline and Doxycycline according to the CLSI standards.

The final output included organism identification and interpreted AST results according to Clinical and Laboratory Standards Institute (CLSI) breakpoints. The system also flagged resistance mechanisms methicillin resistance (MRS), where applicable. All results were reviewed and validated using internal software quality control checks, and any inconsistencies were resolved manually before finalization.

2.4. Phenotypic confirmation of MRCoNS isolates

Phenotypic identification of methicillin-resistant coagulase-negative staphylococci (MRCoNS) was carried out using the cefoxitin disk diffusion assay in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (2020) [19]. Colonies of CoNS obtained from overnight cultures were inoculated into nutrient broth. The suspensions were adjusted to a 0.5 McFarland standard and spread onto Mueller–Hinton agar (Lab M, Lancashire, UK) plates in duplicate, with cefoxitin (30 µg) and oxacillin (1 µg) discs (Mast Diagnostics, UK). Plates were incubated at 37 °C for 24 hours. Isolates exhibiting cefoxitin resistance, defined as a zone diameter of ≤ 21 mm, were classified as MRCoNS.

2.5. Quality control

To ensure the accuracy of bacterial identification and susceptibility testing, standard quality control (QC) strains were tested alongside samples. These included *Staphylococcus aureus* ATCC 25923. QC results were compared against expected performance ranges recommended by CLSI [19]. Deviations beyond acceptable limits would have required repeating the tests.

2.6. Data processing and analysis

All data collected from microbial cultures and antimicrobial susceptibility testing were entered into Microsoft Excel for organization and adequate cleaning. Data were checked for completeness, consistency, and accuracy prior to analysis. Statistical analyses were done using SPSS version 16/frequencies and percentages were computed to summarize the distribution of bacterial isolates, as well as their antimicrobial resistance patterns. Results were presented in tables and graphs to facilitate clear interpretation.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Prevalence of Methicillin Resistance Coagulase-Negative *Staphylococcus* (MR-CoNS)

This study found an 18.2% (26/143) prevalence of MR-CoNS. Of the 26 MR-CoNS isolated, 9/26 (34.6%) were identified as *Staphylococcus schleiferi*. Others included 6/26 (23.0%) *Staphylococcus xylosus*, 4/26 (15.3%) *Staphylococcus cohnii*, 3/26 (11.5%) *Staphylococcus capitis*, 2/26 (7.6%) *Staphylococcus lugdunensis*, and 2/26 (7.6%) *Staphylococcus saprophiticus* as presented in Figure 1. *Staphylococcus schleiferi* was the most prevalent coagulase-negative *Staphylococcus* organism.

3.1.2. Distribution of MR-CoNS by clinical samples analysed

MR-CoNS were isolated mainly from wound/pus swabs as they are a normal part of the skin's microbiome, making them readily available to contaminate or infect wounds, as presented in Table 1. The majority of the MR-CoNS were isolated from wound/Pus swabs.

3.1.3. Antimicrobial susceptibility patterns of the isolated MR-CoNS

All MR-CoNS isolated were 100% sensitive to linezolid, vancomycin, and teicoplanin. *S. cohnii* and *S. schleiferi* showed >90% sensitivity to Moxifloxacin, Monocycline, and Trimethoprim/Sulfamethoxazole. There was notable resistance to daptomycin, which is one of the new antibiotics for the treatment of severe *Staphylococcus* infection. The resistance to gentamycin was 75-100% for all isolates (Table 2).

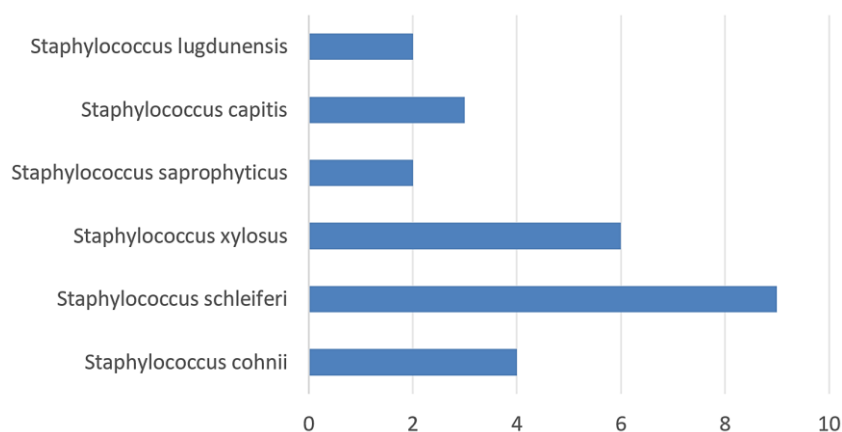


Figure 1. The graph of prevalence of MR-CoNS strains recovered from various clinical samples

Table 1. Distribution of MR-CoNS from clinical samples that were analysed

Sample type	Clinical significance	No of MR-CoNS	MR-CoNS spp
Wound/Pus swabs	Infection	4	<i>Staphylococcus cohnii</i>
		1	<i>Staphylococcus lugdunensis</i>
		2	<i>Staphylococcus xylosus</i>
		2	<i>Staphylococcus capitis</i>
		3	<i>Staphylococcus schleiferi</i>
Urine	Infection	2	<i>Staphylococcus xylosus</i>
		1	<i>Staphylococcus lugdunensis</i>
		3	<i>Staphylococcus schleiferi</i>
High vaginal swab	Infection	1	<i>Staphylococcus saprophyticus</i>
		2	<i>Staphylococcus schleiferi</i>
		1	<i>Staphylococcus saprophyticus</i>
		2	<i>Staphylococcus xylosus</i>
Blood	Infection	1	<i>staphylococcus capitis</i>
Semen	Infection	1	<i>Staphylococcus schleiferi</i>

Table 2. The antimicrobial sensitivity profile of MR-CoNS isolated

Antibiotics	<i>S. Cohnii</i> n = 4		<i>S. Schleifferi</i> n = 9		<i>S. xylosus</i> n = 6		<i>S. saprophyticus</i> n = 2		<i>S. Capitis</i> n = 3		<i>S. lugdunensis</i> n = 2	
	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)
Penicillin	25	75	0	100	0	100	0	100	0	100	0	100
Oxacillin	0	100	0	100	0	100	0	100	0	100	0	100
Trimethoprim/Sulf	100	0	90	10	66.7	33.3	100	0	66.7	33.3	0	100
Clindamycin	100	0	87.5	12.5	33.3	66.7	100	0	66.7	33.3	0	100
Erythromycin	0	100	12.5	87.5	0	100	0	100	0	100	0	100
Gentamicin	25	75	10	90	0	100	0	100	0	100	0	100
Nitrofurantoin	100	0	90	10	33.3	66.7	100	0	66.7	33.3	0	100
Levofloxacin	0	100	20	80	16.7	83.3	0	100	0	100	0	100
Moxifloxacin	100	0	90	10	50	50	100	0	66.7	33.3	0	100
Doxycycline	100	0	90	10	66.7	33.3	100	0	66.7	33.3	50	50
Minocycline	100	0	87.5	0	33.3	66	100	0	100	0	0	100
Vancomycin	100	0	100	0	100	0	100	0	100	0	100	100
Teicoplanin	100	0	100	0	100	0	100	0	100	0	100	100
Tigecycline	50	50	30	70	33.3	66.7	50	50	33.3	66.7	0	100
Linezolid	100	0	100	100	0	0	100	0	100	0	100	100
Rifampicin	50	50	30	70	16.7	83.3	0	100	33.3	66.7	0	100
Daptomycin	100	0	90	10	66.7	33.3	100	0	66.7	33.3	50	50
Clarithromycin	0	100	12.5	87.5	0	100	0	100	0	100	0	100

3.2. Discussion

Antimicrobial resistance (AMR) in bacterial pathogens poses a significant challenge to infection control and prevention efforts. CoNS, which are common commensals of the skin and mucous membranes, share the ecological niche of the anterior nares with *Staphylococcus aureus* and other bacteria [20]–[22]. This proximity facilitates horizontal gene transfer and the exchange of resistance determinants [21]. The situation is further complicated by the emergence of methicillin-resistant CoNS (MR-CoNS). CoNS are increasingly

recognized as reservoirs of resistance traits that can be disseminated across the Staphylococcaceae family [23], including genes conferring resistance to last-resort antibiotics such as linezolid and daptomycin [24], [25].

In staphylococci, methicillin/oxacillin resistance is of particular concern. It is primarily mediated by the *mecA* gene, which encodes an alternative penicillin-binding protein (PBP2a) with reduced affinity for β -lactams. This gene is located on transferable SCCmec genomic elements, mobile genetic structures whose origins have been traced to *Staphylococcus sciuri* (recently reclassified as *Mammaliococcus sciuri*) [26]–[29] and macrococcal species [30].

3.2.1. Prevalence of methicillin resistant coagulase negative *Staphylococcus*

From the clinical samples analyzed, methicillin-resistant coagulase-negative staphylococci (MR-CoNS) were detected at a prevalence of 18.2%. To the best of our knowledge, no published data currently exist on MR-CoNS in Freetown, Sierra Leone. It is also noteworthy that CoNS are often regarded as contaminants in both clinical and non-clinical contexts, a perception that may be widespread across many African countries [31]. Nevertheless, comparable studies conducted in Africa have documented MR-CoNS in human populations [32]–[36], with a pooled prevalence estimated at 36% across the continent [37].

Our study found 9/26 (26.9%) *Staphylococcus schleiferi* as the predominant MR-CoNS. Others included, 6/26 (23.0%) *Staphylococcus xylosus*, 4/26 (15.3%) *Staphylococcus cohnii*, 3/26 (11.5%) *Staphylococcus capitis*, 2/26 (7.6%) *Staphylococcus lugdunensis* and 2/26 (7.6%) *Staphylococcus saprophiticus*. Unlike our study, most studies have found *S. epidermidis* to be the most prevalent MR-CoNS. However, majority if these studies were about colonization and not necessarily infection [38]–[41] as illustrated in Figure 1 and Table 1. *Staphylococcus schleiferi* was recognized in the late 1980s as a new species of CoNS. Some studies have revealed *S. schleiferi* infections to be more common with more than half of the patients with evidence of immunosuppression. The majority of our isolates were from HIV/AIDs MPox coinfecting individuals. Therefore, our study is an agreement with similar studies that found *S. schleiferi* infection in humans, having a possible association with immunosuppression [42], [43]. *Schleiferi* has been shown to be more virulent than, for instance, *Staphylococcus warneri* or *Staphylococcus hominis*. However, a recent review of CoNS and MR-CoNS did not cite *S. schleiferi* among the CoNS that have been isolated. This can be owed to limited diagnostic capacity, but also the notion that all CoNS are contaminants which is a perception prevalent among most clinicians and microbiologists [37].

3.2.2. Antibiotic resistance patterns of the isolated MR-CoNS

Fortunately, we did not find any resistance to newer antibiotics such as linezolid in our sample. Equally vancomycin and teicoplanin exhibited 100% activity against all the MR-CoNS isolated. All isolates showed 50-100% sensitivity to Daptomycin and a 90-100% sensitivity exhibited by *S. cohnii* and *S. schleiferi* to Moxifloxacin, Monocycline, and trimethoprim/sulfamethoxazole, as presented in Table 2. *S. cohnii* showed sensitivity to most antibiotics, while *S. lugdunensis* showed resistance to most of the antibiotics tested. We found a 10%, 33.3%, 33.3%, and 50% resistance to daptomycin by *S. schleiferi*, *S. xylosus*, *S. capitis*, and *lugdunensis*, respectively. Daptomycin is one of the newer antibiotics against staphylococcal infections. Our results are consistent with previous studies that have documented co-resistance and multidrug resistance in CoNS, not only among human isolates but also in samples from animals and environmental sources [44]–[49]. These findings serve as a warning signal, indicating the ongoing introduction and dissemination of multidrug-resistant CoNS (MR-CoNS) into both the hospital and the community settings.

At present, the management of CoNS infections is challenging due to widespread resistance to β -lactams and several other antibiotic classes. Newer agents such as linezolid, daptomycin, and tigecycline may serve as alternative treatment options, provided they are accessible and considered cost-effective. Some MR-CoNS in our study exhibited resistance to the reserve antibiotics such as daptomycin. Whilst this resistance could be intrinsic, it represents a worrying trend as these antibiotics are not currently in use in Sierra Leone. However, their uncontrolled use and natural resistance can accelerate the spread of mobile resistance genes, reinforcing the need for safeguarding these antibiotics.

Our study has several limitations. First, being a single-site purposive study, the findings cannot be readily generalized, as antibiotic resistance and bacterial profiles were characterized only at one location in Sierra Leone. To better understand the burden and resistance patterns of MR-CoNS infections, further investigations across multiple tertiary health facilities are warranted, particularly in the context of hospital-acquired infections. Additionally, MR-CoNS detection in this study was limited to phenotypic characterization. Future research should incorporate molecular and proteomic approaches (e.g., whole genome sequencing, MALDI-TOF) to provide deeper insights into species diversity, SCCmec types, and potential clonal distributions within Sierra Leone. Such data would be valuable for policymakers, infection

prevention and control specialists, and microbiologists in developing effective strategies to mitigate the public health impact of MR-CoNS. Despite these constraints, our study offers the first evidence of MR-CoNS resistant pathogens isolated from clinical samples in Sierra Leone.

4. CONCLUSION

This study highlights the concerning prevalence of resistant MR-CoNS to commonly used antibiotics in the local context of Sierra Leone. The emergence of such resistance patterns poses a significant and growing threat, potentially rendering multiple antimicrobial therapies ineffective for treating MR-CoNS infections. Therefore, practical approaches like standardizing the diagnosis of these infections, improving infection prevention and control practices, enhancing the diagnostic capacity, and instituting antimicrobial stewardship programs will ultimately combat the spread of antibiotic resistance among the MR-CoNS. However, the success of these initiatives relies on sustained resource allocations, capacity building of resourceful personnel, and a good diagnostic bacteriological quality control system.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

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C : **C**onceptualization

M : **M**ethodology

So : **S**oftware

Va : **V**alidation

Fo : **F**ormal analysis

I : **I**nvestigation

R : **R**esources

D : **D**ata Curation

O : Writing - **O**riginal Draft

E : Writing - Review & **E**diting

Vi : **V**isualization

Su : **S**upervision

P : **P**roject administration

Fu : **F**unding acquisition

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

INFORMED CONSENT

Informed consent was sought from each patient before their sample was included in the study.

ETHICAL APPROVAL

Ethical approval was obtained from the scientific research board of Premium Medical Services, and permission sought from the management of Premium Microbiology Laboratory. Confidentiality was ensured with strict storage of data and use of unique identification numbers. All procedures involved

were performed in accordance with the ethical standards and the 1964 Helsinki declarations and their current amendments.

DATA AVAILABILITY

The data supporting this study's findings can be obtained from the corresponding author, [ABM], upon reasonable request.





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