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Inducible Clindamycin Resistance in Gram-positive Cocci Isolated from Clinical Specimens of Patients with Bacterial Infections at a Tertiary Hospital in Tanzania

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ABSTRACT

Background: Clindamycin is a reserved antibiotic used to treat infections caused by Gram-positive cocci; however, increasing bacterial resistance threatens its effectiveness. Routine antimicrobial susceptibility testing may fail to detect inducible macrolide-lincosamide-streptogramin B (iMLS B) resistance, which requires the double disc diffusion (D-test) for accurate identification. Therefore, this study aimed to use the D-test to determine the prevalence of inducible clindamycin resistance among Gram-positive cocci isolates from patients with bacterial infections at a tertiary hospital in Tanzania.

Methods: A cross-sectional study was conducted among patients presenting with bacterial infections at Muhimbili National Hospital (MNH) in Tanzania from April to August 2022. Convenience sampling was used to include all eligible clinical specimens yielding Gram-positive cocci during the study period. All Gram-positive cocci isolated from the participants' clinical specimens were subjected to antimicrobial susceptibility testing (AST) using the Kirby-Bauer disc diffusion method, and the D-test was performed to phenotypically detect iMLS B resistance. Demographic variables (age and sex), clinical specimen types, bacterial species, and antimicrobial resistance profiles were collected from patients' records and laboratory results. Data were analyzed using Stata® Statistical Software version 15.1 (StataCorp LLC, College Station, TX, USA). Descriptive statistics were used to summarize the data, while the Chi-square test was used for analysis of categorical variables. A p-value < 0.05 was considered statistically significant.

Results: A total of 246 Gram-positive cocci isolates from clinical specimens were analyzed. The majority were Coagulase-negative Staphylococci (CoNS) 64.6%, followed by ***Staphylococcus aureus*** 30.1%. The prevalence of inducible clindamycin resistance was 25.2% (95% Confidence Interval (CI) [20.2%-30.9%]). Among the ***Staphylococcus aureus*** and CoNS isolates, 39.2% (95% CI [28.9%-50.6%]) and 20.8%(95% CI [15.2%-27.7%]) exhibited the iMLS B resistance phenotype, respectively. In addition, 63.5% of ***Staphylococcus aureus*** isolates were phenotypically confirmed as methicillin-resistant ***Staphylococcus aureus*** (MRSA), and 44.7% of these isolates demonstrated the iMLS B resistance phenotype. Furthermore, 75.6% (95% CI [69.9%-80.6%]) of the Gram-positive bacterial isolates were multidrug-resistant (MDR).

Conclusions: The present study demonstrated that a substantial proportion of Gram-positive cocci isolates exhibited iMLS B resistance, and the prevalence of MDR was high. These findings highlight the importance of incorporating the D-test into routine antimicrobial susceptibility testing to guide appropriate antibiotic therapy for infections caused by Gram-positive cocci. Furthermore, the results provide baseline evidence for future surveillance studies and support the need for strengthened antimicrobial stewardship programs and continued research to monitor and control antibiotic resistance in resource-limited settings.

BACKGROUND

Gram-positive cocci are spherical bacteria that have a thick, cross-linked peptidoglycan cell wall layer that retains crystal violet stain following Gram staining.[1] Gram-positive cocci include *Staphylococcus* (catalase-positive), which grows in clusters, and *Streptococcus* (catalase-negative), which grows in chains. The staphylococci further subdivide into coagulase-positive (*Staphylococcus aureus*) and coagulase-negative (*Staphylococcus epidermidis* and *Staphylococcus saprophyticus*) species.[1] Streptococcus bacteria subdivide into *Streptococcus pyogenes* (Group A), *Streptococcus agalactiae* (Group B), enterococci (Group D), *Streptococcus viridans*, and *Streptococcus pneumonia*.[1]

Gram-positive cocci, particularly *Staphylococcus* and *Streptococcus* species, are major human pathogens responsible for a wide range of serious infectious diseases globally, particularly in low- and middle-income countries where healthcare resources and infection-control infrastructure are often limited [2]. Pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus* species are responsible for a wide spectrum of infections, including bloodstream infections, pneumonia, skin and soft tissue infections, meningitis, endocarditis, osteomyelitis, and surgical site infections [2]. These infections contribute substantially to global morbidity and mortality and represent a significant public health challenge, particularly in low- and middle-income countries [2-5]. In particular, bloodstream infections (BSIs) caused by Gram-positive cocci carry significant mortality risks, with 30-day mortality rates estimated at 12.5% to 30%, depending on the specific pathogen and patient population.[6]

In addition, Gram-positive cocci are commonly associated with hospital-acquired infections, and some are associated with multidrug resistance (MDR) profiles.[4, 5, 7-10] This is due to the rise in irrational antibiotic use, which has increased selective pressure on bacteria, resulting in the emergence of antimicrobial resistance[11-13].

The macrolides, lincosamides, and streptogramin B (MLSB) group is a crucial alternative class of antibiotics for treating Gram-positive coccus infections. Among the MLSB group, clindamycin has been considered the preferred agent for the management of Gram-positive coccus infections because of its excellent pharmacokinetic properties [14]. Clindamycin has good penetration and distribution into the skin and other soft tissues and acceptable oral absorption with no dosage adjustment in patients with kidney disorders.[15, 16] Despite these properties, increasing clindamycin resistance has been reported worldwide, including in Africa[17]. A systematic review of studies in Africa found an overall prevalence of inducible clindamycin resistance (iMLSB) in *Staphylococci aureus* isolates of 19.8%, with rates ranging from 2.9% to 44.0%; with high prevalence reported in Egypt (44%), followed by Libya (35.8%) and Uganda (33.3%) [17]. MSLB resistance is a bacterial resistance phenotype affecting three antibiotic classes: macrolides, lincosamides, and streptogramin B [18]. Resistance occurs through target-site modification of the bacterial ribosome[18, 19]. Bacteria acquire the *erm* (erythromycin ribosome methylase) genes, which encode an rRNA methyltransferase[18]. This enzyme methylates an adenine residue (A2058) within the 23S rRNA of the 50S ribosomal subunit. Methylation alters the macrolide-lincosamide-streptogramin B binding site, preventing these antibiotics from binding effectively and thereby inhibiting their action on protein synthesis[18, 19]. This resistance exists as either constitutive, where the *erm* gene is expressed continuously, or inducible, where the *erm* gene is expressed only in the presence of a macrolide such as erythromycin[18].

The emergence of iMLS B resistance among Gram-positive cocci has become a major concern because it may not be detected by routine antimicrobial susceptibility testing methods [20]. Several studies conducted in resource-limited settings, including African countries, have reported increasing rates of inducible clindamycin resistance among *Staphylococcus aureus* and coagulase-negative staphylococci[7, 10, 15, 21, 22]. Therefore, given the widespread use of this antibiotic, especially in resource-limited settings, it is essential to monitor clindamycin resistance patterns to guide the selection of appropriate antibiotic treatment and the management of infections caused by Gram-positive cocci[15].

Detection of inducible clindamycin resistance requires the double-disc diffusion test (D-test), which is recommended for inclusion in routine antimicrobial susceptibility testing. However, in many laboratories in resource-limited settings, including Tanzania, the D-test is not routinely performed, leading to limited data on the prevalence of inducible clindamycin resistance [20]. Consequently, monitoring the clindamycin resistance profile among Gram-positive cocci is essential for guiding appropriate antibiotic selection and improving infection management.

Therefore, the present study aimed to use the D-test to provide updated information on the current profile of inducible clindamycin resistance among Gram-positive cocci isolated from clinical specimens of patients attending a tertiary hospital in Dar es Salaam, Tanzania. In the present study, we selected Muhimbili National Hospital (MNH) because it is a national hospital that receives patients from across the country, providing a broad referral base. Hence, bacterial isolates obtained at this facility are often considered representative of antimicrobial resistance patterns observed across different parts of Tanzania. The findings of this study will provide evidence to inform clinicians and other stakeholders on iMLS B resistance patterns and emphasize the importance of incorporating routine D-testing in microbiology laboratories in resource-limited settings.

METHODS

Study site

This study was conducted in the Microbiology unit of the Central Pathology Laboratory (CPL) at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania. MNH is a national referral hospital, research center, and university teaching hospital that provides specialized healthcare services to patients from across the country. The Microbiology unit is where clinical specimens from patients with suspected bacterial infections are processed for culture and antimicrobial susceptibility testing. The Microbiology unit receives approximately 30 specimens per day, averaging about 600 specimens per month for culture and sensitivity testing. The Microbiology unit at CPL, MNH, is accredited according to ISO 15189 standards by the Southern African Development Community Accreditation Service (SADCAS).

Study Design and Study Period

This study employed a hospital-based cross-sectional design to determine the prevalence and profile of inducible clindamycin resistance among Gram-positive cocci isolated from clinical specimens. A cross-sectional approach was chosen because it allows the assessment of antimicrobial resistance patterns among bacterial isolates obtained from patients during a defined period without follow-up. The study utilized routine clinical specimens submitted for culture and antimicrobial susceptibility

testing, and laboratory analyses were performed on Gram-positive cocci isolates recovered from these specimens.

The study was conducted over five months, from April to August 2022, at the Microbiology unit at CPL, MNH, in Dar es Salaam, Tanzania. During this period, all eligible clinical specimens received at the Microbiology unit were processed using standard microbiological procedures.

Study Population, Sampling Procedure, and Sample Size Determination

Study Population

The study population consisted of patients with suspected bacterial infections whose clinical specimens were submitted to the Microbiology unit at CPL, MNH, for culture and antimicrobial susceptibility testing during the study period. The analytical units for this study were Gram-positive cocci isolates recovered from these specimens.

Inclusion Criteria

Patients were included in the study if they had a clinical suspicion of bacterial infection and their clinical specimens (such as blood, pus, sputum, urine, throat swabs, or other body fluids) were submitted to the Microbiology unit, CPL at MNH for culture and antimicrobial susceptibility testing during the study period.

In the present study, a suspected bacterial infection was defined clinically as the presence of signs and symptoms suggestive of a bacterial infection. These included fever (≥38 °C), clinical signs of inflammation such as redness, swelling, warmth, or pain, increased respiratory rate, and other system-specific manifestations consistent with bacterial infection.

Only specimens that yielded Gram-positive cocci isolates, including *Staphylococcus aureus* or coagulase-negative staphylococci (CoNS), and had viable isolates available for antimicrobial susceptibility testing and the D-test for inducible clindamycin resistance were included.

Exclusion Criteria

Specimens were excluded if they showed no bacterial growth, yielded organisms other than Gram-positive cocci, or were duplicate isolates from the same patient during the same episode of infection. Specimens with incomplete clinical or laboratory records were also excluded from further analysis.

Sampling Procedures

A convenience sampling approach was used, whereby all eligible Gram-positive cocci isolates obtained during the study period were consecutively included until the required minimum sample size of isolates was reached. The selection of samples was proportional to the routine sample submissions.

Sample Size Determination

The minimum sample size (N) was calculated using the Kish and Leslie[38] formula for a single population proportion:

$$N = \frac{Z^2 \times P(1-P)}{(d)^2}$$

Assuming a 95% confidence level (Z=1.96), a precision (d) of 5.0%, and an expected prevalence of inducible clindamycin resistance based on previous studies ($P1$=36.5% for *Staphylococcus aureus* and $P2$=3.52% for CoNS), giving an average expected prevalence (P) of 20.01%.[23, 24]

$$P = \frac{\text{P1} + \text{P2}}{2} = \frac{36.5\% + 3.52\%}{2} = 20.1\%$$

$$N = \frac{1.96^2 \times 0.201(1 - 0.201)}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.201 \times 0.799}{0.0025}$$

$$N = 246$$

Thus, the minimum sample size (N) was 246.

Data Collection

Demographic and clinical information, including age, sex, hospital ward/department, and type of specimen, were extracted from patient records, laboratory request forms and the Laboratory Information Management System (LIMS).

Laboratory Procedures

In the present study, each specimen was processed in accordance with Standard Operating Procedures (SOPs). Blood culture samples were incubated in the BD-BACTEC-FX40 system until evidence of presumptive microbial growth was observed. Pus, sputum, urine, and throat swab specimens were processed using standard bacteriological techniques. Thereafter, positive blood culture samples and the other clinical specimens (pus, sputum, urine, and throat swab) were inoculated onto sheep blood agar base (HIMEDIA: M1301-500G), chocolate agar base (HIMEDIA: M103-500G), and MacConkey agar (HIMEDIA: MHD81-500G), and incubated at 37 °C in ambient air for 18 to 24 hours. Following incubation, bacterial growth was examined based on colony morphology. Gram staining was performed on representative colonies to determine Gram reaction and cellular morphology. Isolates showing Gram-positive cocci were further identified using conventional biochemical tests, including catalase and coagulase tests.

The Gram-positive cocci specimens isolated during the study period were subjected to antimicrobial susceptibility testing (AST) using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar.[25] The antimicrobial disks used for AST were erythromycin (15 µg), clindamycin (2 µg), and cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), doxycycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and penicillin (10 µg) (CLSI M100, 2022).[25] AST was followed by the D-test to determine inducible clindamycin resistance in erythromycin-resistant (ER) and clindamycin-susceptible (CLI-S) isolates.[25] All clindamycin-sensitive isolates were subjected to the D-test, except *Enterococcus* species, due to the lack of clinical utility, since clindamycin is not widely used for the treatment of *Enterococcus* species-associated infections and the fact that *Enterococcus* species are known to have intrinsic resistance to lincosamides.[26] Briefly, macrolide disks were placed at distances of 15 mm and 12 mm (edge

to edge) from the clindamycin disk for *Staphylococcus* and *Streptococcus* species, respectively, on plates inoculated with the bacterial suspension with adjusted turbidity using a 0.5 McFarland turbidity standard (*Hardy Diagnostics, Santa Maria, CA, USA*), which corresponds to an approximate bacterial concentration of 1.5×10^8 colony-forming units (CFU)/mL, followed by overnight incubation at 37°C. The results were interpreted as follows: moderate sensitivity (MS) phenotype, sensitive to clindamycin with a circular zone of inhibition around the disk; inducible resistance (IMLSB) phenotype, sensitive to clindamycin with a D shaped zone of inhibition around the clindamycin disk; and constitutive resistance (cMLSB) phenotype, resistant to clindamycin with a circular zone of inhibition.

Methicillin resistance (MR) in *Staphylococcus* species was also detected by the disk diffusion method using a cefoxitin disk as per CLSI M100, 2022.[25] *Staphylococcus aureus* and CoNS resistant to cefoxitin were termed methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant Coagulase Negative Staphylococci (MRCoNS), respectively. *Staphylococcus* spp. susceptible to cefoxitin are termed methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-susceptible coagulase-negative *Staphylococci* (MSCoNS).

Quality Control

Cultures showing evidence of contamination or polymicrobial growth were carefully evaluated by Gram staining and colony morphology. When mixed bacterial populations were observed on primary culture plates, representative colonies with distinct morphology suggestive of Gram-positive cocci were selected and subcultured onto fresh sheep blood agar to obtain pure isolates. The subcultures were incubated at 37 °C in ambient air for 18 to 24 hours. After incubation, colony purity was confirmed by Gram staining. Only pure isolates demonstrating Gram-positive cocci morphology were subjected to further identification using conventional biochemical tests, including catalase and coagulase tests.

Quality control for antimicrobial susceptibility testing and D-test procedures was performed using *Staphylococcus aureus* ATCC 25923 in accordance with CLSI M100, 2022, recommendations.[25]

Study Variables

The independent variables in this study were sociodemographic characteristics (age and sex), hospital ward/department information, and specimen type. The dependent variables were Gram-positive cocci bacteria with inducible clindamycin resistance and MDR bacteria.

In this study, MDR bacteria are defined as bacteria having resistance to at least one antibiotic in three or more antibiotic classes.

Data Analysis

Data were entered and cleaned using Microsoft Excel® 2021 and exported to STATA® statistical software version 15.1 (StataCorp LLC, College Station, TX, USA) for descriptive analysis. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables are presented as frequencies and percentages. Differences in socio-demographic features and inducible clindamycin resistance were determined using the chi-square test and considered significant at a P value <.05.

Ethical Considerations

The present study obtained ethical clearance to conduct the study from the Institutional Review Board (IRB) of the Muhimbili University of Health and Allied Sciences (MUHAS), with reference number DA.282/298/01L/Reg. No. 2019-04-13777. Permission to conduct the study was requested from the Executive Director of MNH, Tanzania. In this study, we received a waiver of participant consent from the Senate Research and Publications Committee (SRPC), MUHAS, because our study involved the analysis of clinical and laboratory data from routine samples collected for diagnostic purposes; there was no direct patient contact. Participants' information was handled with confidentiality and privacy. During data extraction, all personal identifiers such as patient names, hospital identification numbers, and other identifiable information were removed from the dataset. Each record was assigned a unique study code to allow data management and analysis without revealing patient identity. The anonymized dataset was used for all analyses, and access to the data was restricted to the research team. Thus, the study's findings cannot be linked to any particular patient.

RESULTS

Description of Gram-positive Cocci Isolated From Clinical Specimens of Study Participants

A total of 246 participants' clinical specimens that yielded Gram-positive cocci were processed during the study period. The majority (79.7%) of clinical specimens were blood, followed by pus (11.8%), sputum (6.5%), urine (0.8%), other body fluids (0.8%), and throat swab (0.4%). The specimens from the male patients accounted for 51.2% of all specimens. The majority (88.2%) of clinical specimens were from inpatients, with specimens from the Intensive Care Unit (ICU) accounting for 13.4%. The characteristics of the study participants are summarized in Table 1. Among the 246 Gram-positive cocci isolated and identified in this study, 64.6% were CoNS, followed by 30.1% Staphylococcus *aureus* (Figure 1).

FIGURE 1: Distribution of Isolated Gram-positive Cocci

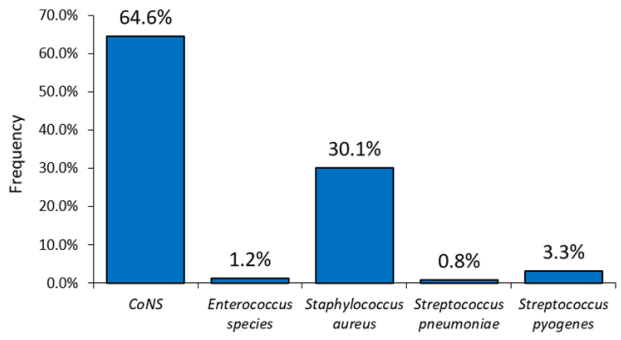


TABLE 1: Study Participants' Characteristics (N=246)

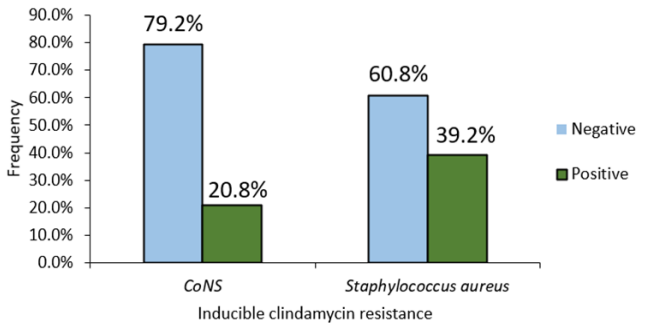
Characteristics	Number of participants	Positive D-Test	Negative D-Test	*P value*
Age (years)				
0-10	31 (12.6)	6(19.4)	25(80.6)	.382
11-21	18 (7.3)	7 (38.9)	11(61.1)	
22 - 32	56 (22.8)	11(19.6)	45(80.4)	
33 - 43	78 (31.7)	22(28.2)	56(71.8)	
>44	63 (25.6)	16(25.4)	47(74.6)	
Sex				
Female	120 (48.7)	29(24.2)	91(75.8)	.823
Male	126 (51.2)	33(26.2)	93(73.8)	
Clinical specimen				
Blood	196 (79.7)	51(26.0)	145(74.0)	.601
Pus	29 (11.8)	9(31.0)	20(69.0)	
Sputum	16 (6.5)	2(12.5)	14(87.5)	
Urine	2 (0.8)	0(0)	2(100)	
Throat swab	1 (0.4)	0(0)	1(100)	
Other body fluids	2 (0.8)	0(0)	2 (100)	
Department/ward				
Inpatient wards	217 (88.2)	57 (26.3)	160 (73.7)	.718
Outpatient department	29 (11.8)	5 (17.2)	24(82.8)	

D-test indicates the Double-disk diffusion test for the detection of inducible clindamycin resistance; p-value is the Chi-square test

Prevalence of Inducible Clindamycin Resistance Among Gram-positive Cocci Isolates

All Gram-positive cocci isolates were subjected to antimicrobial susceptibility testing, and clindamycin sensitive isolates were subjected to a D-test for the detection of inducible clindamycin resistance. In the present study, the prevalence of inducible clindamycin resistance was 25.2% (95% Confidence Interval:20.2%-30.9%) and was only detected in *Staphylococcus* species. Among the *Staphylococci* spp, inducible clindamycin resistance was detected in 39.2% (95% CI:28.9%-50.6%) of the *Staphylococci aureus* isolates and 20.8% (95% CI: 15.2%-27.7%) of the CoNS isolates (Figure 2).

FIGURE 2: Proportion of Inducible Clindamycin Resistance among *Staphylococci spp*



In the present study, 53.2% of the isolates with inducible clindamycin resistance were obtained from male patients.

The distribution of inducible clindamycin resistance in isolates according to the department/hospital wards where specimens were collected revealed that the majority (91.9%) of inducible clindamycin-resistant isolates were from the inpatient wards, where the majority (21.1%) were from the Intensive Care Unit (ICU). The majority of inducible clindamycin resistance isolates were obtained from blood specimens (82.3%), followed by pus specimens (14.5%) and sputum (3.2%).

In this study, 63.5% (95% CI: 52.1%-73.6%]) of *Staphylococcus aureus* isolates were phenotypically confirmed as MRSA. Among the isolated MRSA 44.7%, had inducible clindamycin resistance, whereas only (29.6%) of MSSA had inducible clindamycin resistance. In contrast, 23.1% and 8% of MRCoNS and MSCoNS, respectively, had inducible clindamycin resistance. Table 2 summarizes the frequency of inducible clindamycin resistance among the *Staphylococcal* species.

Antimicrobial Resistance Pattern Among Gram-positive Cocci Isolated from Clinical Specimens

In this study, the antimicrobial resistance pattern among Gram-positive cocci revealed that all *Staphylococcus aureus* isolates were resistant to penicillin (100%), and 93.0% of the CoNS were resistant to penicillin. All (100%) *Streptococcus* species were resistant to trimethoprim-sulfamethoxazole. Specifically, 50% of *Streptococcus pyogenes* isolates were resistant to macrolides, 50% to clindamycin, and 57% to doxycycline. In this study, 50% of *Streptococcus pneumoniae* isolates were resistant to macrolides and clindamycin. *Enterococcus* species were resistant to macrolides (66.7%), clindamycin (66.7%), and ciprofloxacin (66.7%) (Table 3). In addition, we assessed the proportion of multidrug-resistant (MDR) Gram-positive cocci. We observed that approximately 75.6% (95% CI:69.9%-80.6%) of the Gram-positive bacterial isolates were MDR, with the majority at 27.4%. of the bacterial isolates being CoNS.

TABLE 2: Proportion of Inducible Clindamycin Resistance Among *Staphylococci spp*

Inducible Clindamycin Resistance	Methicillin susceptibility n (%)			
	MRSA	MSSA	MRCoNS	MSCoNS
Negative	26 (55.3)	19(70.4)	103(76.9)	23(92.0)
Positive	21(44.7)	8 (29.6)	31(23.1)	2 (8.0)
Total	47(100.0)	27(100.0)	134(100.0)	25(100.0)

MRSA = Methicillin-Resistant *Staphylococcus aureus*; MSSA = Methicillin-Susceptible *Staphylococcus aureus*; MRCoNS = Methicillin-Resistant Coagulase-Negative *Staphylococci*; MSCoNS = Methicillin-Susceptible Coagulase-Negative *Staphylococci*

TABLE 3: Antimicrobial Resistance Pattern for Gram-positive Cocci Isolated from Clinical Specimens at MNH, Tanzania

Antibiotic	CoNS n=159	*S. aureus* n=74	*S. pyogenes* n=8	*S. pneumoniae* n=2	*Enterococcus spp* n=3
Cefoxitin	133(84.2)	48(64.9)	-	-	-
Erythromycin	138(86.8)	48(64.9)	4(50.0)	1(50.0)	2(66.7)
Clindamycin	38(23.9)	20(27.0)	4(50.0)	1(50.0)	2(66.7)
Chloramphenicol	14(14.6)	9(20.2)	1(25.0)	-	0(0.0)
Ciprofloxacin	71(51.5)	35(50.7)	1(100.0)	1(100.0)	2(66.7)
Gentamycin	98(69.0)	31(45.6)	-	-	-
Doxycycline	25(17.9)	15(22.4)	4(57.1)	-	0(0.0)
Trimethoprim sulfamethoxazole	95(67.9)	29(43.9)	7(100.0)	-	1(100.0)
Penicillin	63(93.0)	42(100.0)	3(75.0)	-	-

CoNS = Coagulase-Negative *Staphylococci*; *S. aureus* = *Staphylococcus aureus*; *S. pyogenes* = *Streptococcus pyogenes*; *S. pneumoniae* = *Streptococcus pneumoniae*; CLSI = Clinical and Laboratory Standards Institute

DISCUSSION

Macrolide resistance is a growing clinical challenge, especially in developing countries. This study assessed the prevalence of inducible clindamycin resistance among Gram-positive cocci isolated from clinical specimens at a tertiary hospital in Dar es Salaam, Tanzania.

Our findings revealed that inducible clindamycin resistance was present in 25.2% (95% CI: 20.2%-30.9%) of Gram-positive cocci isolates. The prevalence of inducible clindamycin resistance observed in this study is similar to findings reported in other low and middle income settings, such as a study conducted in Ethiopia that reported a prevalence of 26.3% among *Staphylococcus aureus* isolates.[27] Our findings are also comparable to those reported in a systematic review conducted in Africa, which reported the highest prevalence of inducible clindamycin resistance among *Staphylococcus aureus* isolates in clinical settings ranged from 30% to 44.0%, with countries such as Egypt, Uganda, and Libya having the highest prevalences; while countries such as Kenya had prevalence from 26% to 29%.[17] Furthermore, another study conducted in Nepal among 1027 clinical samples reported a prevalence of 36.5%.[28] Our findings further indicate that the frequency of inducible clindamycin resistance among Gram-positive cocci is high, which raises concerns about the use of macrolide-lincosamide-streptogramin B antibiotics in *Staphylococcus aureus* infections. Hence, the D-test should be performed as part of routine testing to detect inducible clindamycin resistance and prevent treatment failure.

In this study, inducible clindamycin resistance was detected exclusively among *Staphylococcus* species, particularly *Staphylococcus aureus* and CoNS, at prevalences of 39.2% (95% CI: 28.9%-50.6%) and 20.8% (95% CI: 15.2%-27.7%), respectively. However, the prevalence of inducible clindamycin resistance in CoNS in this study was lower than that reported in another study conducted in Iraq, which reported a prevalence of 40%.[29] The difference could be due to differences in sample size, as the study in Iraq had a small sample of 28 CoNS isolates compared with our study, which had 159 isolates. In addition, laboratory methods for isolation and detection differed, whereby the study in Iraq used both conventional techniques and the Vitek®2 system.[29] Nevertheless, these findings indicate that inducible clindamycin resistance is frequently present in both *Staphylococcus aureus* and CoNS in most settings.

The distribution of inducible clindamycin resistance

observed in this study indicated a higher proportion among isolates obtained from inpatient wards, particularly from the ICU, and most resistant isolates were recovered from blood specimens, which accounted for 79.7% of all specimens. This observation differs from that reported in Iran, where inducible clindamycin resistance was also frequently observed in inpatient participants, but was higher among those admitted to internal medicine (medical) wards and was more frequent in urine samples than in blood samples, despite blood samples being the majority. This suggests that the predominance of inducible clindamycin resistance among isolates from ICU patients and blood specimens in this study differs from reports in other settings, indicating that local epidemiological and healthcare factors may influence the pattern of inducible resistance rather than the mere predominance of a particular specimen type.

However, these findings should be interpreted cautiously. The present study analyzed routine laboratory isolates rather than collecting clinical data directly from patients; therefore, it was not possible to determine whether infections were hospital-acquired or community-acquired. Therefore, this limits the ability to draw conclusions regarding the epidemiological source of the infections.

The present study reported 63.5% (95% CI: 52.1%-73.6%]) of *Staphylococcus aureus* isolates were phenotypically confirmed as MRSA, and 44.7% had inducible clindamycin resistance, whereas only 29.6% of MSSA had inducible clindamycin resistance. These findings are consistent with previous reports from Iran, India, and Nepal, where higher rates of inducible clindamycin resistance were observed among MRSA isolates.[4, 22, 30-33] The higher prevalence of inducible clindamycin resistance among MRSA may be explained by the presence of *erm* genes, particularly *ermA* and *ermC*, which encode methylases that confer resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics. These genes are often carried on mobile genetic elements such as the transposon *Tn554*, facilitating their dissemination among resistant *Staphylococcal* strains.[34] This observation could also be attributed to differences in the proportions of MRSA and MSSA, with MRSA being more frequent.[4, 33]

Interestingly, inducible clindamycin resistance was not detected among the *Streptococcus* species isolated in this study, including *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In contrast, a study conducted among paediatric patients in Israel reported inducible clindamycin resistance in *Streptococcus* species, with constitutive MLSB resistance as the predominant mechanism.[35] Differences between these findings may be attributed to variations in study populations; in the study in Israel, the main population was children, who may have had different antimicrobial exposure patterns. Furthermore, in this study, they analysed only *Streptococci* spp. isolates, suggesting that circulating bacterial strains across different geographical regions could explain the resistance pattern.

Nevertheless, these findings highlight the continued importance of routine detection of inducible clindamycin resistance among Gram-positive cocci in clinical microbiology laboratories using the D-test to avoid therapeutic failure during treatment with clindamycin.

Furthermore, the antimicrobial resistance pattern observed in this study demonstrated high resistance to penicillin among *Staphylococcus aureus* isolates, consistent with previous reports indicating widespread penicillin resistance in *Staphylococci* spp. due to β-lactamase production.[21,36] In addition, we observed that approximately 75.6% (95% CI: 69.9%-80.6%) of bacterial isolates were MDR, consistent with findings reported elsewhere in similar resource-limited settings, highlighting the growing burden of antimicrobial resistance in hospital settings.[37]

From a public health perspective, the relatively high prevalence of inducible clindamycin resistance and MDR observed in this study has important clinical implications. Failure to detect inducible resistance may lead to inappropriate use of clindamycin and subsequent treatment failure when relying on empirical treatment.[37] Therefore, it is essential to enforce the use of the D-test in clinical microbiology laboratories, particularly in tertiary hospitals where resistant pathogens are more common.

CONCLUSION

In the present study, 25.2% of Gram-positive cocci, particularly *Staphylococcus aureus* and CoNS isolates, exhibited inducible clindamycin resistance and were multidrug-resistant. These findings underscore the importance of routinely implementing the D-test in clinical microbiology laboratories for isolates that demonstrate erythromycin resistance and clindamycin susceptibility, in accordance with CLSI recommendations, to accurately detect inducible clindamycin resistance and prevent potential treatment failure. In addition, strengthening antimicrobial stewardship programs and Infection Prevention and Control (IPC) measures is essential to reduce the emergence and spread of multidrug-resistant pathogens.

Future studies should focus on molecular characterization of resistance genes such as *erm* genes responsible for MLSB resistance, as well as multicenter surveillance studies to better understand the epidemiology and distribution of inducible clindamycin resistance across different healthcare settings in Tanzania and similar settings.

Limitations

The present study had several limitations that should be considered when interpreting the findings. First, the study was conducted in a single tertiary hospital, which may limit the generalizability of the results to other healthcare settings or regions. Second, we used convenience sampling, which could introduce selection bias, since the included isolates were limited to patients who presented to the hospital. This further limited the ability to determine the clinical context of infections, including whether they were hospital-acquired or community-acquired. We also used a cross-sectional study design, which also limits the evaluation of temporal changes. We were also unable to assess prior antibiotic use that could have affected the MDR patterns we observed. In addition, our study relied on phenotypic detection of inducible clindamycin resistance using the D-test, and molecular characterization of resistance genes was not performed; therefore, specific genetic mechanisms underlying the

observed resistance phenotypes could not be determined. Finally, the relatively small number of isolates for some bacterial species, such as *Streptococcus pneumoniae* and Enterococcus species, may limit the interpretation of resistance patterns in these organisms.

Nevertheless, since the study was conducted at a national hospital, we believe our findings could be representative, inform the burden of clindamycin resistance, and provide baseline data. Future studies incorporating molecular techniques, larger sample sizes, and multicenter data would provide a more comprehensive understanding of inducible clindamycin resistance among Gram-positive pathogens.

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