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Inducible clindamycin resistance in coagulase negative staphylococci

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***Abstract*---**Induced clindamycin resistance poses a major problem for the use of this drug to treat staphylococcal infections. An infectious etiology is often implicated in the presence of CoNS in clinical specimens. A total of 183 isolates of Staphylococci spp. recovered from different clinical specimens were studied. A coagulase test was done to select coagulase negative Staphylococci, and they were confirmed by conventional microbiological techniques. Using the double disc approximation test, induced clindamycin resistance was detected (D-test). The D test was used to detect the inducible clindamycin resistance phenotype in erythromycin resistant and clindamycin sensitive isolates. A micro titer plate test was used to determine biofilm development in these isolates. One hundred and twenty eight isolates from various clinical specimens were identified as proven CoNS, which were distributed across six different species'. haemolyticus, the most common species of CoNS isolated, was isolated from all types of specimens. The present study revealed the strong association of biofilm with inducible clindamycin resistance among the CoNs isolates.

***Keywords*---**Clindamycin resistance, Erythromycin resistant, Biofilm, D–test.

Introduction

Coagulase-negative staphylococci (CoNS) are Gram-positive cocci that distribute in uneven "grape-like" bunches and are distinguished from S. aureus by their incapability to yield coagulase and coagulate rabbit plasma. In the past, coagulase-negative staphylococci were thought to be nonpathogenic[1]. However, in recent years, their importance as pathogens, as well as their growing prevalence, has come to light. Coagulase-negative staphylococci are a significant portion of the normal microbiota, and they also inhabit mucous membranes in adults and children from a few weeks of age[2]. CoNS (such as S. epidermidis, S. capitis, S. hominis, S. warneri, and S. haemolyticus), while normally thought of as skin commensals, are the most common endemic nosocomial pathogens in neonates.
It's important to note that the clinical presentations of patients with CoNS infections are often different from those of patients with Staphylococcus aureus infections, which are more common in patients on corticosteroid therapy, patients undergoing hemodialysis, and patients with implanted catheters or prosthetic valves [3] This is a relatively new finding, and particular variables implicated in pathogenesis are currently being investigated. There is also a tendency for CoNS to be drug-resistant to a wide range of multiple drug classes .[4] It has long been known that S. aureus is more susceptible to antimicrobial agents such as lactam antibiotics, whereas CONS is less susceptible. Macrolide antibiotic resistance in CoNS may be due to an active efflux mechanism encoded by msrA (conferring resistance to macrolides and type B streptogramins only) or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins (MLSB resistance) [5]

It has long been known that S. aureus is more susceptible to antimicrobial agents such as lactam antibiotics, whereas CONS is less susceptible. Resistance to inducible MLSB cannot be detected using traditional susceptibility test methods, such as broth-based or agar dilution susceptibility testing. Failure to identify inducible MLSB resistance may result in the failure of clindamycin treatment in the clinical setting [6].

A typical antimicrobial susceptibility testing method, such as a standard disc diffusion test or an E-test, or a standard broth-based susceptibility test, cannot detect inducible MLSB resistance. Strains with inducible clindamycin resistance show erythromycin resistant and clindamycin sensitive in antimicrobial susceptibility testing [7] Strains articulating inducible MLSB resistance can be sensed by a simple test, recognized as the Disk approximation test or D-zone test, where a flattened inhibition zone near the clindamycin disc (positive ‘D-zone test’) when erythromycin and clindamycin discs are placed close to each other [8]. Tests for erythromycin (ERY) and clindamycin (CC) disc approximation were first proposed by Fiebelkorn et al., and are presently designated in the Clinical and Laboratory Standards Institute’s (CLSI) antimicrobial susceptibility brochures. This has made the discovery of inducible CC resistance in staphylococci a simple test for clinical microbiology laboratories to accomplish, particularly if they have previously carried out disc diffusion assessments [9]. Biofilm obstructs the distribution of antibiotics and contributes to antibiotic resistance. Current studies have supported that CoNs have precise features and an antimicrobial susceptibility pattern. At the present, the frequency and physical characteristics

of biofilm development in CoNs with induced clindamycin resistance have not been revealed [10].

Hence, the present study was undertaken to assess the occurrence of inducible clindamycin resistance among Staphylococcus spp. using the D test. The drive of this study was to portray the antimicrobial susceptibility patterns and to assess, according to the D test. In this study, the biofilm formation of the CoNs was also determined and compared.

Materials and Methods

Study design and Isolation

The study was conducted from February to May 2020. A total of 183 isolates of *Staphylococcus Sps.,* isolated from clinical specimens. The clinical samples (n = 127) were from urine, infected blood, catheter tip, tissue samples, wound culture, respiratory samples, nasal samples, body fluids, ear swabs, urethral discharge, abscess fluid, cerebro-spinal fluid, semen, and cervical swabs. All samples except urine samples were cultured on MacConkey agar, and Blood agar plates, incubated aerobically at 37^{o}C for 24 hours[11].

Species identification

Staphylococcal colonies were identified by Gram staining, catalase and coagulase test.Further speciation was done by bloos method and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using various antibiotics as per CLSI guidelines.

Antibiotics susceptibility and D-test

The D-test was used to detect inducible resistance in clindamycin-susceptible and erythromycin-resistant (ER-R) isolates.CLSI (Clinical Laboratory Standard Institute 2009) instructions recommended for an inoculation and incubation on Muller-Hinton agar (MHA) plate with a 0.5-McFarland equivalent solution. MHA plates were covered with discs of Clindamycin and Erythromycin, spaced 15-26mm apart from each other.Plates were examined after 18 hours of incubation at 37°C. For clindamycin resistance, flattening of the inhibitory zone (D-shaped) was investigated [12]. The interpretation was done as follows:

- Growth up to CLI and ERY discs designates resistance to both ERY and CLI (cMLS $_B$ phenotype).
- Formation of compressed CLI zone amid ERY and CLI disc shows inducible clindamycin resistance, (iMLS $_B$ phenotype).
- No flattening of CLI zone - negative for inducible clindamycin resistance (MS phenotype) i.e. resistant to ERY but susceptible to CLI.

Phenotypic analysis of biofilm formation

For biofilm detection, the Microliter Plate Assay (MTP) was used as reported before.Briefly, overnight cultures of bacteria were incubated in TSB (Merck) that included 1 present glucose, and then diluted to 1:100 with new media. After incubation for 24 hours at 37°C in sterile MTP with polystyrene flat bottoms and

200 µL diluted culture, the colonies were discarded. After incubation, wells were washed three times with 200 µL of phosphate-buffered saline (pH 7.2) to remove planktonic bacteria. Afterward, wells were fixed by 99% methanol, dried at room temperature, and then stained with 0.1% crystal violet. Dye bound to the adherent cells was dissolved with 1 mL of 95% ethanol per well. The optical density (OD) of each well was measured using a micro titre plate reader at a wavelength of 490 nm. Optical density cut-off defined as average OD of negative control + 3 × standard deviation of the negative control. Biofilm formation of strains was analysed according to the absorbance of the crystal violet-stained attached cells and interpreted as per the criteria described by Stepanovic et al . Accordingly, the degree of biofilm production was categorized into strong, moderate, weak, or without biofilm [13]

Results and Discussion
Distribution of coagulase negative Staphylococcus

Based on biochemical tests, 183 Staphylococcal isolates were isolated from varying clinical specimen, of which 128(69.9%) were identified as proven CoNS that distributed among 6different species. Of the fifteen samples sources, the most common source of CoNS species was blood culture (n = 57, 44.5%) isolates, followed by abscesses (n=36, 28.1 %), catheter tip (n = 34, 23.4%), urine (n=28, 21.8%) and wound (n=19, 14.8 %) (Figure 1). Analysis of each individual isolated CoNS showed a variable distribution among the different sources of samples (Figure 2).*S. haemolyticus,* the most common species of CoNS isolated, was isolated from all types of specimens. The second most commonly isolated CoNS species was *S. epidermidis.*The majority of these bacteria were isolated also identified to be in high presence on catheter tips and in urine specimens. The species level distribution was depicted in Figure 2.

Inducible clindamycin resistance among the isolates based on D test

Routine disc diffusion testing was used to determine the sensitivity of all 128 Staphylococcal isolates to erythromycin and other antibiotics in the panel; 29 of them were resistant to erythromycin.It was shown that 13 (10.1%) of the isolates tested positive for the D-test, which indicates constitutive resistance due to the presence of the MLSB Phenotype, whereas the remaining 28 isolates tested negative for the D-test.Table 1 shows the detailed distribution. In order to study the development of biofilm, MTP was employed. Both subjectively and statistically, $cMLS_B$ strains produced more biofilm compared to susceptible bacteria.When a biofilm was strong (+++), it meant there was a higher chance of antibiotic resistance or tolerance, which might lead to treatment failures.Furthermore, seventeen isolates of (58.6%) CoNS were found to be biofilm producers among the 29 D test positive strains.

CoNS have a wide range of methods for infecting and surviving in their host. Since they play an important role in the natural flora, CoNS have mainly been considered contaminants.It's been suggested in some recent research that these organisms are responsible for a large number of human illnesses, notably in immune-compromised individuals and preterm babies.CoNS isolates were detected in blood in a number of different species, which is in line with earlier

comparable investigations.As in earlier investigations, *S. haemolyticus* was the most common isolate, followed by *S. epidermidis*[14].Our outcomes are reliable with studies done in other countries.

Aside from blood cultures being the most prevalent source of CoNS species separation, our data demonstrate that alternative sources such as catheter tips and urine cultures can also be used to isolate CoNS.Hospitals' growing usage of central venous catheters (CVCs) and other indwelling vascular devices may result in higher infection rates as a result. Perhaps the high number and proportion of isolated CoNS isolates and infected catheter tip cultures might be attributed to skin contamination of staff and patients[15].

When treating infected individuals, the antimicrobial susceptibility of a clinical isolate is typically essential. As resistance increases and multidrug resistant organisms evolve, this is of particular importance. The increasing frequency of staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of CL therapy to treat such infections. For the treatment of Staphylococcal infections, there are various choices, with clindamycin being one of the better ones. When it comes to staphylococcal isolates with inducible phenotype, clindamycin resistance has been observed in both in vitro tests as well as in vivo after clindamycin treatment. In CoNS resistance to macrolides (e.g. erythromycin), lincosamides (e.g. clindamycin) and type B streptogramins (MLSB) can be the result of ribosomal target change in which enzymes encoded by erm genes confer constitutive or inducible resistance to MLS drugs through methylation of the 23S rRNA. The MSRS genes in staphylococci can also encode an active efflux system that confers resistance to MLS B alone, but not to lincosamides, allowing the bacteria to evade antibiotics. As a result of conventional susceptibility testing, isolates with constitutive resistance can be easily identified.Clindamycin may be active against staphylococci with inducible clindamycin resistance when tested using conventional techniques, and the disk- diffusion induction test is used to identify this form of resistance.

The D-test is an easy test to perform along with routine susceptibility testing. The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible clindamycin resistance is helpful in guiding anti-staphylococcal therapy. According to our D test, 29 of the CoNs had iMLSB phenotype in our investigation, but in previous studies, only 20 – 25 percent of the CoNs had iMLSB. In our study, the rate of inducible clindamycin resistance found was very high in comparison to the rates reported in previous studies by Ansari et al. (12.4%) and Adhikari et al. (10%) in Nepal.This might be attributed to differences in Staphylococcus spp. resistance trends across different patient groups, hospitals, time periods, and geographical regions[16].

Geographically, the prevalence of iMLSB resistance in these isolates varies from 7 to 100 percent according to published research [17]. Clindamycin R was developed in 1969 by McGehee and colleagues in vivo and in vitro against ER-resistant staphylococci [18] Other authors have established the prompt *in vitro* of inducible to constitutive MLS_B resistance in staphylococci .In a study conducted in Brazil, 11.3% of *S. aureus* and 13.7% of CoNS isolates were confirmed to have the

inducible MLS_B resistance phenotype. [19] When the ER-R CL-S phenotype is found, several studies suggest that the failure to identify inducible clindamycin resistance may lead to clinical failure.

Approximately 13% of the isolates in this research had inherent clindamycin resistance.This trend is in difference with other reports from Korea where the constitutive resistance (cMLSB) was 32 %.Thus, the prevalence of constitutive and induced resistance in staphylococcal isolates is very variable by hospital and geographical area. The low constitutive clindamycin resistance in our study may also be recognized to the fact that drug is not commonly used and hence there is less selection of resistant strains.

The capacity of CoNS to attach to surfaces has been linked to biofilm development and pathogenicity, with the virulence of the organism varying with its ability to adhere. There is a correlation between antibiotic resistance and the structure of the biofilm, including its robustness and its components. So, we wanted to examine the biofilm development amongst the strains to see how they differed. 58.5 percent of the CONS were extremely pathogenic and had high levels of adhesion. In our study, higher rates of multidrug resistance were found among biofilm producing strains in comparison to biofilm non-producing strains. These findings were in favor of the results reported by Ghasemian et al [20] As a result, the antibiotics may not penetrate the biofilm, bacteria grow slowly, and antibiotic degrading mechanisms may be present. The development of biofilms also provides a platform for horizontal gene transfer between bacteria, resulting in the dissemination of drug resistance markers and other virulence factors. An Algerian study also found extremely adherent, moderately adherent, and non-adherent strains [21]Our results were in line with theirs. It has been observed that Gram-positive bacteria that form biofilms are more resistant to erythromycin, cotrimoxazole, and ciprofloxacin. One study from Nepal found that staphylococcal drug resistance developed owing to the overuse of the antibiotics erythromycin and cotrimoxazole for both mild and more serious staphylococcal infections.

Conclusion

Multidrug resistance, as well as methicillin resistance, is more likely to occur in biofilm-positive species as compared to biofilm-negative species. Consequently, there is a high risk of impaired treatment and dissemination of infection, increasing the morbidity and mortality of the admitted patients. Frequent monitoring of biofilm development and antibiotic resistance profiles in CONS isolates is recommended. Our understanding of these factors might help us design an effective antibacterial policy for the early treatment of infection.

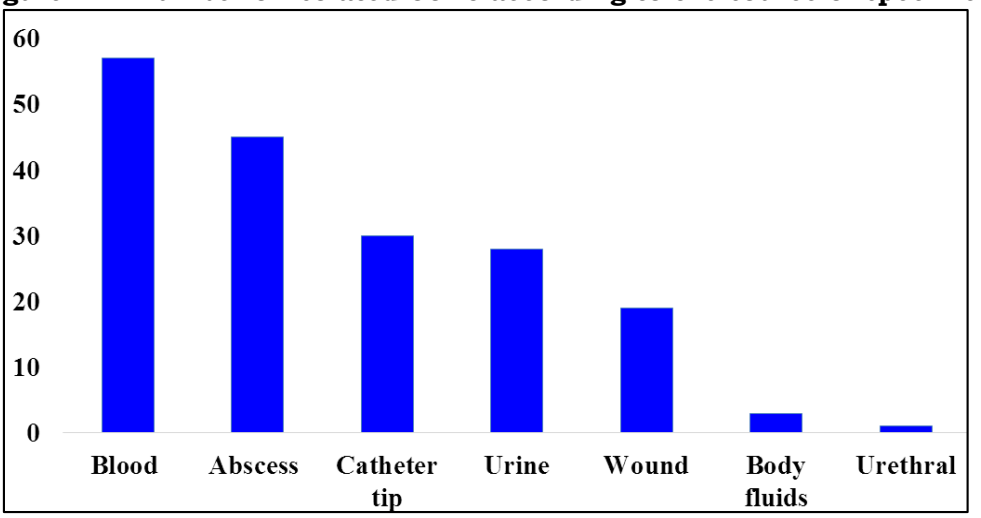
References

1. Becker K, Heilmann C, Peters G,2014.“Coagulase-negative staphylococci”. Clinical microbiology reviews. 27(4),870-926.
2. Michalik M, Samet A, Podbielska-Kubera A, Savini V, Międzobrodzki J, Kosecka-Strojek M, 2020. “Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: a review”. Annals of Clinical Microbiology and Antimicrobials. 19(26)1-0.

3. Ma XX, Wang EH, Liu Y, Luo EJ,2011. ‘’Antibiotic susceptibility of coagulase-negative staphylococci (CoNS): emergence of teicoplanin-non-susceptible CoNS strains with inducible resistance to vancomycin. Journal of medical microbiology.”1 60(11),1661-8.
4. Chajęcka-Wierzchowska W, Zadernowska A, Nalepa B, Sierpińska M, Łaniewska-Trokenheim Ł,2015.“Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin–phenotypic and genotypic antibiotic resistance.” Food microbiology.1(46),222-6.
5. Becker K, Both A, Weißelberg S, Heilmann C, Rohde H, 2020.“Emergence of coagulase-negative staphylococci.” Expert review of anti-infective therapy.18(4),349-66.
6. De Oliveira A, Cataneli Pereira V, Pinheiro L, MoraesRiboli DF, Benini Martins K, da Cunha RD, De Lourdes M,2016.“Antimicrobial resistance profile of planktonic and biofilm cells of Staphylococcus aureus and coagulase-negative staphylococci”. International journal of molecular sciences.17(9), 1423.
7. Deyno S, Fekadu S, Seyfe S,2018.“Prevalence and antimicrobial resistance of coagulase negative staphylococci clinical isolates from Ethiopia: a meta-analysis”. BMC microbiology. 18(1), 1-1.
8. Soares LC, Pereira IA, Pribul BR, Oliva MS, Coelho SM, Souza M, 2012.“Antimicrobial resistance and detection of mecA and blaZ genes in coagulase-negative Staphylococcus isolated from bovine mastitis”. Pesquisa Veterinária Brasileira. 32(8), 692-6.
9. Michels R, Last K, Becker SL, Papan C,2021. “Update on Coagulase-Negative StaphylococciWhat the Clinician Should Know”. Microorganisms. 9(4),830.
10. Kürekci C.,2016.“Prevalence, antimicrobial resistance, and resistant traits of coagulase-negative staphylococci isolated from cheese samples in Turkey”,Journal of dairy science. 99(4), 2675-9.
11. Dibah S, Arzanlou M, Jannati E, Shapouri R,2014.“Prevalence and antimicrobial resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from clinical specimens in Ardabil, Iran.” Iranian journal of microbiology. 6(3),163.
12. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T,2005.“Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure”. Antimicrobial agents and chemotherapy. 49(3),1222-4.
13. Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić S, Ćirković I, Ruzicka F,2007.“Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci”. Apmis. 115(8),891-9.
14. McManus BA, Coleman DC, Deasy EC, Brennan GI, O’Connell B, Monecke S, Ehricht R, Leggett B, Leonard N, Shore AC,2015. “Comparative genotypes, staphylococcal cassette chromosome mec (SCCmec) genes and antimicrobial resistance amongst Staphylococcus epidermidis and Staphylococcus haemolyticus isolates from infections in humans and companion animals”. PloS one. 10(9). e0138079.
15. Ohara-Nemoto Y, Haraga H, Kimura S, Nemoto TK, 2008.“ Occurrence of staphylococci in the oral cavities of healthy adults and nasal–oral trafficking of the bacteria”. Journal of Medical Microbiology. 57(1),95-9.

16. Pal N, Sharma B, Sharma R, Vyas L,2010.“Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India”. Journal of postgraduate medicine. 56(3),182.
17. Sun X, Lin ZW, Hu XX, Yao WM, Bai B, Wang HY, Li DY, Chen Z, Cheng H, Pan WG, Deng MG, 2018. “Biofilm formation in erythromycin-resistant Staphylococcus aureus and the relationship with antimicrobial susceptibility and molecular characteristics”. Microbial pathogenesis. 1(124),47-53.
18. RF RM, Barre FF, Finland M.1968.“Resistance of Staphylococcus aureus to lincomycin, clinimycin, and erythromycin”. Antimicrobial agents and chemotherapy. 8, 392-7.
19. Chaudhary NKand Piya R,2021. “Macrolide-Lincosamide-Streptogramin B Resistance Among *Staphylococcus aureus* In Chitwan Medical College Teaching Hospital, Nepal”. Asian Journal of Pharmaceutical and Clinical Research.14(5), 61-5.
20. Ghasemian A, Peerayeh SN, Bakhshi B, Mirzaee M. 2016, “Comparison of biofilm formation between methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus ”.Iranian biomedical journal.20(3),175.
21. Lotfi GH, Hafida HA, Nihel KL, Abdelmonaim KH, Nadia AI, Fatima NA, Walter ZI,2014.“Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen”. African Journal of Bacteriology Research. 6(1),1-6.

“Figure 1” – Number of isolated CoNS according to the source of specimens



“Figure 2” - Percentage of isolated CoNS species.

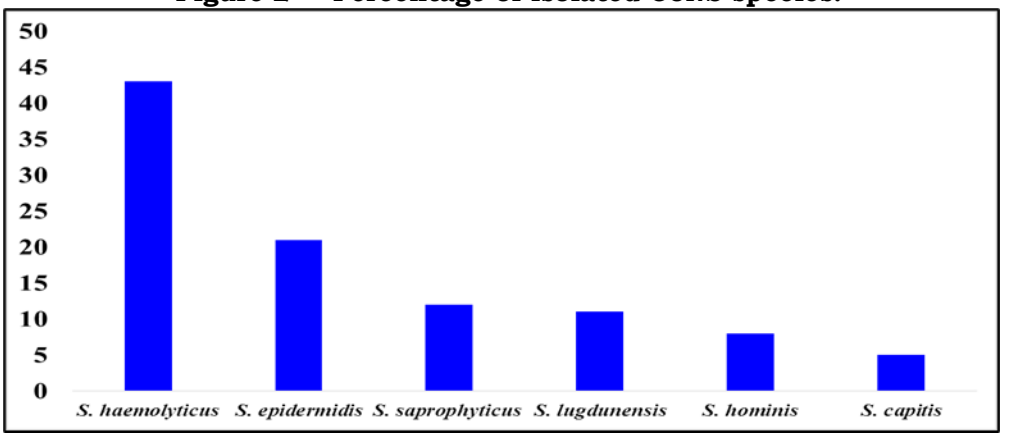


Table 1 - Inducible clindamycin resistance among strains of CoNS

Resistant and susceptible phenotypes	Erythromycin	Clindamycin	D test	Total CoNS (n = 128)
Inducible MLS_B	R	S	+	29
Constitutive MLS_B	R	R	-	13
MS_B	R	S	-	28
Susceptible	S	S	-	58

Table 2 - % of biofilm formation with CoNs isolates

Resistant and susceptible phenotypes	% of Strongly adherents	% of moderate adherents	% of weakly adherents
Inducible MLS_B	58.6	20.6	20.6
Constitutive MLS_B	46.1	30.7	23.07
MS_B	21.4	42.8	35.7
Susceptible	7.25	32.7	62.0