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Original article

Comparative Assessment of D-Test and VITEK® 2 Compact System for Identifying Inducible Clindamycin Resistance in Coagulase-Negative Staphylococci

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Abstract

Background: Coagulase-negative staphylococci (CoNS), once considered commensals, are increasingly recognized as important nosocomial pathogens with significant antimicrobial resistance. Clindamycin is commonly used for treating staphylococcal infections; however, inducible clindamycin resistance mediated by MLSB mechanisms can lead to therapeutic failure if not detected routinely.

Aim: This study aimed to determine the prevalence of inducible clindamycin resistance among nasal carrier CoNS isolates and to compare phenotypic detection by D-test with the automated VITEK-2 Compact system, along with genotypic detection of the ermB gene.

Materials and Methods: Sixty-nine preclinical dental students were examined for nasal colonization by staphylococci. A total of 60 coagulase-negative staphylococci (CoNS) isolates were obtained and identified through conventional microbiological techniques and the VITEK 2 system. Antimicrobial susceptibility results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. Inducible clindamycin resistance was assessed using the D-test, and PCR analysis was conducted to screen for the ermB gene.

Results: Of the 60 CoNS isolates, 45 were methicillin-resistant. Inducible MLSB phenotype was detected in 4 (6.7%) isolates, all belonging to MRCoNS. Constitutive MLSB resistance was observed in 2 (4.4%) MRCoNS isolates. No MLSB resistance was detected among methicillin-sensitive CoNS. The VITEK-2 system showed complete concordance with D-test results. None of the isolates harbored the ermB gene.

Conclusion: The study demonstrates a low prevalence of inducible clindamycin resistance among nasal carrier CoNS, with good agreement between D-test and VITEK-2. Routine screening for inducible resistance remains essential to ensure effective antimicrobial therapy.

Keywords*: Coagulase-negative staphylococci; Inducible clindamycin resistance; D-test; VITEK-2 system; MLSB resistance*

Introduction:

Coagulase Negative Staphylococci (CoNS), traditionally regarded as mere contaminants in clinical cultures, have now been increasingly recognized as significant nosocomial pathogens, especially in hospitalized and immunocompromised patients.[1,2,3] These organisms are a frequent cause of bloodstream infections, infections due to indwelling devices and can contribute to concurrent infections.[4,5,6] The pathogenic potential of CoNS is largely attributed to their ability to form biofilms on foreign bodies, evade host immune responses, and acquire multiple antibiotic resistance determinants.[7,8,9]

Among the limited antimicrobial options available for staphylococcal infections, clindamycin is widely used owing to its excellent tissue penetration, ability to inhibit toxin production, and good oral bioavailability, making it a favorable choice in both community- and hospital-acquired infections.[10,11] However, a significant challenge in the clinical use of clindamycin is the phenomenon of inducible resistance mediated by methylation of the 23S rRNA target site, usually encoded by erm (erythromycin ribosomal methylase) genes.[12,13,14]. These genes can remain silent under routine susceptibility testing conditions and are only expressed in the presence of macrolide inducers such as erythromycin, leading to inducible macrolide-lincosamide-streptogramin B (iMLSB) resistance.[15,16,17]

Failure to detect inducible clindamycin resistance in CoNS can result in therapeutic failure despite apparent in vitro susceptibility, with serious clinical implications including prolonged infection, increased morbidity, and higher healthcare costs.[18,19] Conventional antimicrobial susceptibility testing without dedicated induction assays may not reliably identify these isolates, emphasizing the need for targeted screening strategies.[20] The erythromycin-clindamycin disc approximation test, or D-test, and automated systems with inducible resistance detection modules have therefore become essential tools in routine clinical microbiology laboratories to accurately identify iMLSB phenotypes.[21,22.23]

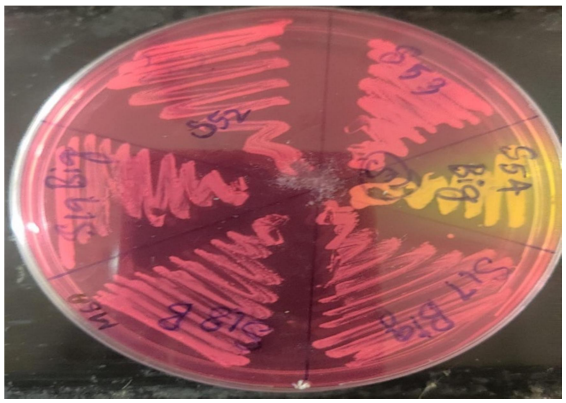
Given the rising incidence of methicillin-resistance and limited antibiotic alternatives, accurate detection of inducible clindamycin resistance among CoNS is critical for effective antimicrobial stewardship and optimal patient management. This study evaluates the frequency of inducible clindamycin resistance in CoNS and also compares automated and conventional phenotypic detection methods.

Materials and methods:

The study included 69 preclinical dental students (14 males and 55 females) of a dental teaching institution at Chennai. Students of both sexes with nil respiratory infections/nasal surgery, rhinitis nor skin and soft tissue lesions in the preceding three months, or use of nasal medications or antimicrobial therapy within two months prior to sampling were enrolled. Ethical approval was obtained from the Institutional Ethics Committee of Sree Balaji Dental College & Hospital, Chennai (Ref. No: SBDCH/IEC/06/2021/1), and samples were collected from consented participants.

Anterior nasal swabs were collected using sterile saline-moistened cotton swabs and transported immediately to the microbiology laboratory. Samples were inoculated into nutrient broth with sodium chloride and sub cultured onto 5% sheep blood agar and MacConkey agar. Staphylococci were identified by Gram staining and standard biochemical tests, including catalase, oxidase, OF, and coagulase tests. Presumptive identification of *Staphylococcus aureus* was performed on mannitol salt agar [Figure 1].

Figure 1: Staphylococcus species on Mannitol salt agar (Mannitol fermenting yellow colonies – *Staphylococcus aureus* , Mannitol non fermenting pink colonies- Coagulase negative staphylococcus after 24 hours incubation at 37°C)



Species identification and antimicrobial susceptibility testing were carried out using the VITEK-2 system (bioMérieux), and results were interpreted according to CLSI 2024 guidelines.[24] Amplification of *ermB* gene if

present was achieved by Multiplex PCR and amplicons visualised using standard gel electrophoretic analysis at 135v, 15 min cycle. The expected product size was 442bp.[25]

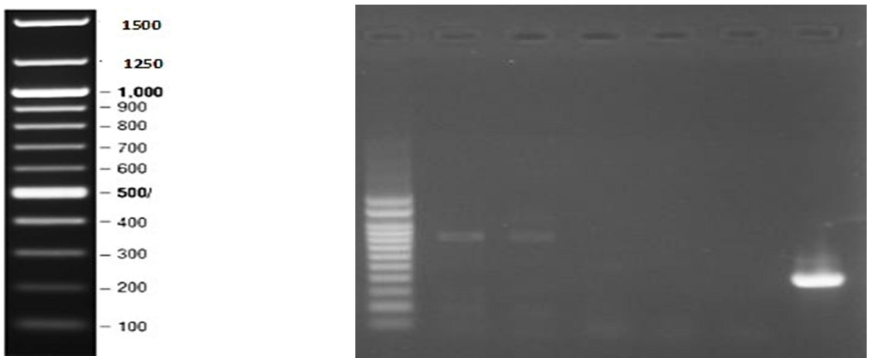
Results:

Of the 69 dental students screened, 55 (13 males and 42 females) were nasal carriers of Staphylococcus species. From these carriers, 60 coagulase-negative staphylococcal (CoNS) isolates were recovered, with dual isolates detected in 7 students. VITEK-2 identification showed *S. epidermidis* as the predominant species (45%), followed by *S. hominis* (23.3%), *S. haemolyticus* (21.7%), *S. lugdunensis* (8.3%), and *S. warneri* (1.7%). Methicillin-resistant CoNS were more common among females than males (77.8% vs. 66.7%), though the difference was not statistically significant ($p = 0.4935$), and no significant gender-based differences were observed across major CoNS species ($p > 0.05$).

Out of 60 CoNS isolates, 45 were methicillin-resistant CoNS (MRCoNS) and 15 were methicillin-sensitive CoNS (MSCoNS). Among MRCoNS isolates, inducible MLS_B phenotype was observed in 4 (8.9%) isolates (Figure 2), while constitutive MLSB phenotype was seen in 2 (4.4%) isolates. No inducible or constitutive resistance was observed among MSCoNS isolates. Overall, inducible clindamycin resistance was detected in 4 (6.7%) CoNS isolates by D-test. Vitek-2 system showed complete agreement with D-test results, with all D-test positive isolates being correctly identified by the automated system. Genotypic analysis by multiplex PCR demonstrated that none of these isolates harbored the *ermB* resistance gene (Figure 3).

Figure 2:D-shaped inhibition zone adjacent to erythromycin disc indicating inducible MLSB phenotype (positive D-test

Figure 3: Gel image of *ermB* gene:



Ladder Specification

442bp for *ermB* gene

Discussion

The present study evaluated the MLSB resistance phenotypes among coagulase-negative staphylococci (CoNS) isolated from nasal carriers. Resistance to erythromycin and clindamycin among MRCoNS was observed at rates of 64.44% and 73.33%, respectively. These findings are lower than those reported in a study from Nepal involving healthcare workers, which documented erythromycin and clindamycin resistance rates of 96.2% and

92.4% among MRCoNS.[26] In contrast, Ishore *et al* has reported a lower erythromycin resistance rate of 58.3%. Such differences highlight the influence of geographic location, antimicrobial usage patterns, and population characteristics on resistance profiles.[27]

Clindamycin being the choicest drug against CoNS infections, particularly when alternative treatments are limited. However, its clinical utility is compromised by MLS_B resistance mechanisms. In the present study, inducible MLS_B ($iMLS_B$) resistance was detected in 8.9% of MRCoNS isolates, while no $iMLS_B$ phenotype was observed among MSCoNS. This prevalence is markedly lower than the reports shown elsewhere, with 45.5%, 43%, 49.2%, and 51.7% of $iMLS_B$ phenotypes among MRCoNS.[28,26,30,29] The reduced frequency of $iMLS_B$ in the present study may reflect lower selective pressure due to restrained antibiotic use or regional differences in the distribution of *erm* genes.

Constitutive MLS_B (cMLSB) resistance was identified in 4.4% of MRCoNS isolates, with no $cMLS_B$ phenotype detected among MSCoNS. This finding contrasts with earlier studies have reported higher $cMLS_B$ prevalence among MRCoNS, which include 13.7% by Supriyarajvi (2015), 20.3% by Raiza Khatoon (2018), 25.8% by Bansal et al. (2012), 27.7% by Kalbhor et *al* (2019), and 50% by Thapa *et al* (2016).[31,30,29,28,26] Additionally, previous studies documented $cMLS_B$ prevalence among MSCoNS ranging from 11.8% to 43%. The low occurrence of $cMLS_B$ resistance in the present study suggests a limited presence of constitutively expressed *erm* genes in this population.

The MS phenotype was not detected in either MRCoNS or MSCoNS isolates in the present study. This finding differs from earlier reports by Thapa *et al*. (2016), Kalbhor *et al*. (2019), Bansal *et al*. (2012), Raiza Khatoon (2018), and Supriyarajvi (2015), who reported MS phenotype prevalence among MRCoNS of 7.1%, 9.1%, 12.4%, 18.6%, and 19.60%, respectively, and among MSCoNS of 22%, 45.16%, 27.3%, 33.3%, and 26.31%.[26,28,29,30,31] The absence of the MS phenotype may indicate limited circulation of *msrA*-mediated efflux mechanisms in this setting.

A noteworthy observation in the present study was the high proportion of isolates susceptible to both erythromycin and clindamycin, accounting for 86.6% of MRCoNS and 100% of MSCoNS. This susceptibility rate is substantially higher than that reported by Kalbhor *et al* (2019), who observed combined susceptibility in only 14.29% of MRCoNS and 12.90% of MSCoNS, suggesting a comparatively favourable antimicrobial susceptibility profile among the CoNS isolates studied.[28]

Molecular analysis revealed that none of the CoNS isolates carried the *ermB* gene. A similar absence of *ermB* was reported by Brzychczy-Wloch *et al.* (2013) among clinical isolates.[32] In contrast, Goudarzi *et al.* (2016) detected *ermB* in 5% of nasal carriers, while Gatermann *et al.* (2007) and Zmantar *et al*. (2011) reported prevalences of 2.3% and 11.1%, respectively, among clinical isolates.[33,34,35] These variations underscore the geographic and population-dependent distribution of *ermB*. The complete absence of *ermB* in the present study suggests that alternative resistance mechanisms, such as *ermA*, *ermC*, or efflux-mediated pathways, may predominate in this region.

Conclusion:

The study shows a low prevalence of inducible and constitutive clindamycin resistance among coagulase-negative staphylococci from nasal carriers, with a high proportion of isolates remaining susceptible to erythromycin and clindamycin. None of the isolates carried the *ermB* gene, indicating regional variation in MLS_B resistance mechanisms. Despite the low frequency of resistance phenotypes, routine D-test screening remains essential to ensure appropriate therapy and prevent treatment failure.

Limitations of the Study:

Being a mono- centric evaluation with fewer samples, least generalization was encountered.

Only nasal carriage isolates from preclinical dental students were included; clinical isolates from active infections were not assessed.

Molecular analysis was limited to detection of the *erm*B gene; other resistance determinants such as *erm*A, *erm*C, and msrA were not evaluated.

Lack of clinical correlation prevented assessment of treatment outcomes associated with inducible resistance phenotypes.

Future Scope:

Multicentric studies with larger and more diverse populations, including clinical isolates, are required to better understand regional resistance patterns.

Comprehensive molecular profiling of MLS_B resistance genes (*ermA, ermC*, msrA) would provide deeper insight into resistance mechanisms.

Longitudinal surveillance studies could help monitor emerging trends in inducible clindamycin resistance.

Integration of routine D-test screening with antimicrobial stewardship programs may improve therapeutic outcomes and prevent treatment failures.

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