

DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG COAGULASE NEGATIVE STAPHYLOCOCCUS ISOLATED FROM HEALTHY POPULATION

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ABSTRACT:

Clindamycin is an attractive agent for empirical therapy for various infections because of its excellent pharmacokinetic and pharmacodynamic properties. Clinical failures of clindamycin therapy for treatment of MRSA infections have been documented for strains that were clindamycin sensitive but erythromycin resistant. The failures were due to inducible resistance to clindamycin. A sum of 20 normal flora isolates of Coagulase-negative staphylococci (CoNS) were subjected to antibiotic sensitivity pattern followed by inducible clindamycin resistance test. 20% were found to be D test positive strains. The D-test is a simple & reliable method to detect inducible & constitutive clindamycin resistance in routine clinical diagnosis setting.

Keywords: *Coagulase-negative staphylococci (CoNS), inducible clindamycin resistance.*

I. INTRODUCTION:

Coagulase-negative staphylococci (CoNS) are part of the normal flora of human skin.

These organisms have relatively low virulence but are increasingly recognised as agents of clinically significant infection of the bloodstream and other sites.[1] Risk factors for CoNS infection include the presence of

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foreign devices (such as intravascular catheters) and immune compromise. Treatment of CoNS infections has become a challenging task due to its multitude of resistance to various antibiotics. Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms: efflux due to macrolide streptogramin resistance (*msrA* gene) and ribosome alteration due to erythromycin ribosome methylase (*erm* gene). [2]

Clindamycin is utilized as a part of the treatment of skin and delicate tissue diseases, brought on by the staphylococcal and Enterococcal species. Great oral retention makes this medication a vital alternative in outpatient treatment or as a follow-up after intravenous treatment. Clindamycin is likewise utilized as an option for patients who are sensitive to penicillin.[3]

Imperviousness to macrolides (e.g. erythromycin) can happen by two unique instruments: efflux due to macrolide streptogramin resistance (*msrA* quality) and ribosome change because of erythromycin ribosome methylase (*erm* quality). [4] Thus, this study indented to detect inducible clindamycin resistance among Coagulase Negative Staphylococcus (CoNS) isolated from healthy population.

II. MATERIALS & METHODS:

Collections of CoNS:

20 samples were collected from different body sites such as anterior nares of nose and fore arm using saline moistened sterile cotton swabs and were seeded onto Blood agar and Mannitol Salt agar (MSA). Isolates were characterized by standard biochemical tests and confirmed.

Antibiotic sensitivity testing:

This has been done using routinely used different antibiotics such as Penicillin, Erythromycin, Clindamycin, Ciprofloxacin, Tetracyclin, Cotrimoxazole and Linezolid by Kirby-Bauer disc diffusion method.[5]

Detection of inducible clindamycin resistance:

Isolates which were resistant to erythromycin were further subjected to 'D test' as per CLSI guidelines. CoNS isolates were made into suspension and turbidity has been matched with 0.5 McFarland standard. These bacterial suspension were lawn cultured on Mueller Hinton agar (MHA). After a brief drying erythromycin (15 mcg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 mcg) disc and was incubated at 37 °C overnight. Flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance. Three different phenotypes were appreciated after testing and interpreted as follows:

1) MS Phenotype - Isolates exhibiting resistance to erythromycin (zone size $\leq 13\text{mm}$) while sensitive to clindamycin (zone size $\geq 21\text{mm}$) and giving circular zone of inhibition around clindamycin was labelled as MS phenotype.

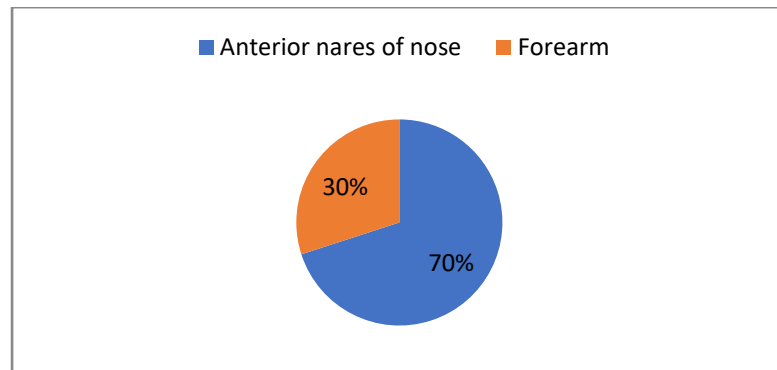
2) Inducible MLS B Phenotype - Isolates showing resistance to erythromycin (zone size $\leq 13\text{mm}$) while being sensitive to clindamycin (zone size $\geq 21\text{mm}$) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.

3) Constitutive MLSB Phenotype - this phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size $\leq 13\text{mm}$) and clindamycin (zone size $\leq 14\text{mm}$) with circular shape of zone of inhibition if any around clindamycin.[6].

III. RESULTS:

Sample wise distribution of Coagulase negative Staphylococcus (CoNS):

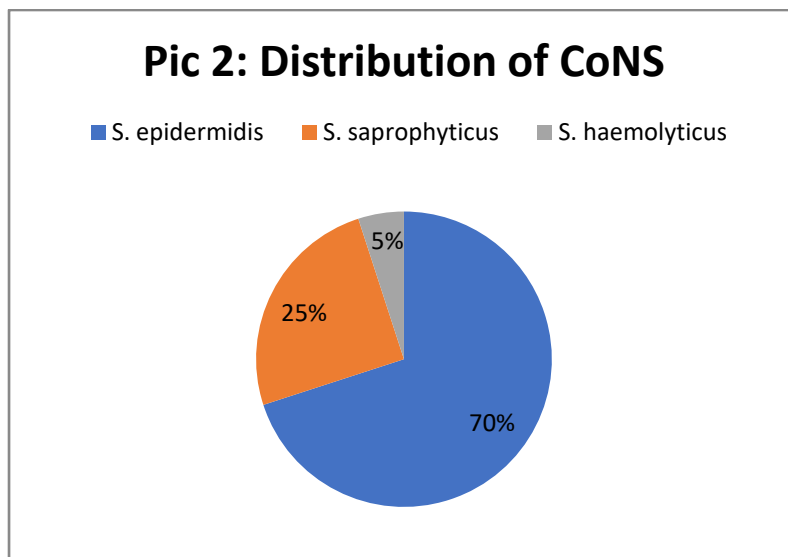
Of the 20 isolates of *CoNS*, 14/20 (70 %) isolates were from anterior nares of nose, 6/20 (30%) from forearm region.



Pic 1:Sample wise distribution of Coagulase negative Staphylococcus (CoNS)

Distribution of CoNS:

Of the 20 CoNS isolates, 14/20 (70%) were found to be *S. epidermidis*, 5/20 (25%) and 1/20 (5%) were belong to *S. saprophyticus* and *S. haemolyticus* respectively.



Antibiotic sensitivity pattern result:

We have observed, total resistance (100%) to penicillin. For erythromycin and clindamycin, 10% of these isolates were shown to be resistant. Complete sensitivity has been demonstrated to linezolid. The detailed results of antibiotic sensitivity pattern to our isolates was shown in table 1.

ANTIBIOTICS	SENSITIVE(%)	INTERMEDIATE(%)	RESISTANT(%)
Penicillin	0	0	20 (100)
Erythromycin	12 (60)	2 (10)	6 (30)
Clindamycin	14 (70)	4 (20)	2 (10)
Ciprofloxacin	9 (45)	6 (30)	5 (25)
Tetracyclin	17 (85)	2 (10)	1 (5)
Cotrimoxazole	12 (60)	5 (25)	3 (15)
Linezolid	20 (100)	0	0

Table 1: Antibiotic sensitivity pattern to CoNS

Results of inducible clindamycin resistance:

CoNS isolates were subjected for susceptibility to erythromycin and other group of antibiotics by the Kirby-Bauer disc diffusion method. Of the 20 isolates, 6 (30%) of them were erythromycin resistance. Detailed results of inducible clindamycin resistance was shown in Table 2.

Clindamycin resistance	Total (n=20)
ERY-S, CL-S	8 (40%)
ERY-R, CL-R	5 (25%)
ERY-R, CL-S(D-test +ve, iMLS)	4 (20%)
ERY-R, CL-S (D-test -ve, MS)	3 (15%)

Table 2:Showing results of clindamycin resistance among CoNS isolates

IV. DISCUSSION:

Medication vulnerability information of the infecting organism is a fundamental figure settling on fitting helpful choices. The variety of components, which give imperviousness to MLS anti-microbials, mirrors the unpredictability of the safe phenotypes and additionally the clinical circumstance. The most boundless and clinically critical resistance components experienced with Gram-positive life forms are the creation of methylases and efflux proteins. The clinical disappointment of clindamycin treatment has been accounted for some time recently. [7,8,9] Hence, there is a need to distinguish the instruments that present imperviousness to MLS anti-infection agents concerning clindamycin treatment of staphylococcal contaminations.

Clindamycin is used in the treatment of skin and soft-tissue infections, caused by staphylococcal species. Good oral absorption makes this drug an important option in outpatient therapy or as a follow-up after intravenous therapy. Clindamycin is a good alternative for the treatment of both methicillin-resistant and susceptible staphylococcal infections. Clindamycin resistance can develop in staphylococcal isolates with the inducible phenotype, and spontaneous constitutively resistant mutants. This study demonstrates that the D shape of the Clindamycin zone adjacent to an Erythromycin disc in a conventional disc diffusion test can serve to detect S. epidermidis or CoNS strains with inducible resistance to Clindamycin.

Among the 20 CoNS isolates studied, 100% showed resistance to penicillin, which was higher than reported in literature 98% [1]. 10% were resistant to Erythromycin and clindamycin, while other studies showed a higher rate of resistance 51% and 33% respectively. [1]. Ciprofloxacin showed 25% with a higher resistance of 37% in studies conducted by others [2]. Tetracycline had 5% resistance. Cotrimoxazole had a lower resistance of 15%, while other studies showed 27% resistance. Linezolid was the most sensitive with 0% resistance, with respect to literature. [1,2].

40% of the isolates were sensitive to both Erythromycin and Clindamycin. 20% of the isolates were iMLSB phenotype with D test positive while 15% were D test negative. 25% were MLSB phenotype.



Pic 3: Representative picture showing D zone of inducible clindamycin resistance

V. CONCLUSION

The clinician must have a wide knowledge of inducible clindamycin resistance and report to laboratory immediately for prompt treatment. The D-test is a simple & reliable method to detect inducible & constitutive clindamycin resistance in routine clinical diagnosis setting.

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