Incidence of macrolide lincosamidestreptogramin B resistance in Coagulase Negative Staphylococci from a tertiary care hospital in Nepal

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ABSTRACT

Coagulase negative Staphylococci (CoNS) are increasingly isolated from clinical specimen. Macrolide, lincosamide and streptogramin B (MLS_B) antibiotics are regaining their importance in the context of methicillin resistance. This study was conducted to find the incidence of different phenotypes of MLS_B resistance among the CoNS isolated from clinical samples over a year (July 2013-June 2014). Eighty three consecutive isolates of CoNS were tested for methicillin resistance using cefoxitin disc and MLS_B resistance phenotypes by erythromycin and clindamycin disc approximation test (D-Test). Resistance to erythromycin was observed in 55 (61.1%) isolates. Of these, 35 (63.63%) had constitutive MLS_B resistance ($cMLS_B$) ; 2 (3.6%) had inducibleMLS_B resistance ($iMLS_B$) while 18 (32.72%) showed MS_Bresistance phenotype. Out of the 26 methicillin resistant CoNS (MR-CoNS), 19 (73.7%) were $cMLS_B$resistant; none were $iMLS_B$ and 6 (23.07%) were MS_B resistant phenotype. In our hospital, clindamycin can be a safe choice for the treatment of infections due to bothmethicillin sensitive and methicillin resistant CoNS which are erythromycin resistant and clindamycin sensitive. D-test should be routinely performed to detect theCoNS with $iMLS_B$ resistant phenotype.

Keywords: Macrolide-lincosamide-streptogramin B resistance, Coagulase negative staphylococcus, Nepal

INTRODUCTION

Coagulase negative Staphylococci (CoNS) have been historically regarded as a harmlessnormal flora with low pathogenic potential.[1,2] However, they are now responsible for serious infections especially in immunocompromised patients, associated with indwelling biomaterials like catheters, prosthetics etc., leading to bloodstream infection.[3] Drug resistant strains pose therapeutic challenge and also act as potential source of resistance genes for pathogens like *Staphylococcus aureus*.[4]

Increasing frequency of methicillin resistance has renewed interest in MLS_B antibiotics. However, their widespread use has led to a large no of staphylococcal strains resistant to these antibiotics.[5]Resistance to MLS_B antibiotics occur by many different mechanisms. Target modification mediated by the *erm* genes is the most common mechanism. They code for methylase enzyme which methylates and alters the target site of MLS_B antibiotics i.e the 23S ribosomal RNA.[6] This can be constitutive ($cMLS_B$) or inducible ($iMLS_B$).[7] The $cMLS_B$ confers resistance to all the MLS_B antibiotics whereas the $iMLS_B$ phenotypes are resistant to 14- membered (e.g., erythromycin, clarithromycin) and 15- membered macrolides (e.g., azithromycin) since they are themselves effective inducers of methylase synthase; reisistance to 16-membered macrolides (e.g.,spiramycin), lincosamide (e.g., clarithromycin) and streptogramin B arise only in the presence of an inducer (erythromycin).[8,9] Spiramycin, a 16- membered macrolide was also shown to have some inducing properties.[10]The *mrs* gene encode the active efflux pump and lead to resistance to macrolides (usually 14- and 15- membered) and streptogramin B. These MS_B resistant phenotypes are sensitive to clindamycin. However, resistance to clindamycin may arise via other mechanisms like enzymatic modification of antibiotics.[10]The MLS_Bresistance phenotypes can be easily distinguished by the erythromycin- clindamycin disc approximation test (D-test).[9]The sensitivity of D test performed at 15-20mm disc spacing was 100% when compared with*erm* and *mrs* gene detection by polymerase chain reaction (PCR).[9,10,11]

This study was conducted to investigate the rate of MLS_B resistance and its phenotypes in both the methicillin sensitive (MR-CoNS) and methicillin resistant CoNS (MR-CoNS) isolated in our hospital since this information is useful in therapy guidance and this data from Nepal is conspicuously lacking.

MATERIALS AND METHODS

Consecutive, non-repeat isolates of CoNS from clinical samples processed in the microbiology laboratory of NepalMedical College Teaching Hospital, Kathmandu, Nepal over a period of one year (July 2013- June 2014) were included in this study. Standard microbiological procedures were followed for the isolation and

identification of CoNS.[12]Methicillin resistance was detected using the cefoxitin (30µg) disc following the Clinical Laboratory Standard Institute guidelines.[13] MLS_B resistance was tested using the D-test.[14]Briefly, 5-6 colonies of the bacteria from blood agar were suspended tomatch with 0.5McFarland's turbidity standard. Lawn culture from this suspension was made on Mueller Hintonagar plate. Erythromycin (15µg) and clindamycin (2µg) discs were placed at a distance of 15mm edge to edge. Readings were taken after incubating these plates aerobically at 35˚C for 18 hours.

Strains resistant to erythromycin and clindamycin were noted as $cMLS_B$ phenotype. Those erythromycin resistant strains sensitive to clindamycin but with flattening of zone of inhibition adjacent to erythromycin (D-zone) were considered $iMLS_B$ phenotype and those without this flattening as the MS_B resistant phenotype.

RESULTS

Eighty three CoNS isolates from various clinical specimens were studied. MR-CoNS were 26 (31.3%). Erythromycin resistance was detected in 55 (61.1%) isolates. D test performed on these demonstrated most strains as $cMLS_B$ resistant phenotype (table 1). The distribution of MLS_B phenotypes among the MR-CoNS and MS-CoNS is shown in table 2.

DISCUSSION

Out of the 83 CoNS isolates, erythromycin resistance was observed in 55 (61.1%).Lincosamides (clindamycin) are generally not recommended for the treatment of infections by erythromycin resistant *Staphylococcus* spp. as they share a similar mode of action and have a common resistance mechanism. However, as there are multiple mechanisms of resistance and diverse phenotypic expression of resistance, clindamycinmaystill have a role in eradicating strainstruly susceptible to clindamycin (the MS_B resistant phenotype).[15,16]Therefore, D-test was performed on the erythromycin resistant isolates which identified three distinguishable phenotypes: $cMLS_B$, $iMLS_B$ and MS_B. The $cMLS_B$ resistance phenotype was observed in 35 (63.63%). This high rate may be a reflection of the increased use of erythromycin/ clindamycin. This phenotype is detectable without the disc approximation test. Strains with $cMLS_B$ resistance are resistant to all MLS antibiotics.

Among the erythromycin resistant CoNS,$iMLS_B$reistance phenotype was observed in only 2 (3.6%) isolates, both from MS-CoNS. This is a reassuring finding since, clinically, $iMLS_B$ resistant bacterial strains have a high rate of spontaneous mutation to constitutive resistance and the use of non inducer antibiotics like clindamycin can lead to selection of constitutive mutants at frequencies of 10^{-7} cfu[17, 18] culminating in failure of clindamycin/ lincomycin therapy.[16] However, clindamycin may still be effective in some patients as the infection is eradicated before the development of resistance.[15]

Clindamycin is a useful alternative for penicillin allergic patients for the treatment of skin and soft tissue infections by methicillin sensitive and resistant strains of staphylococci. Except the central nervous system, it has excellent tissue penetration, accumulates

Table 1: Distribution of MLS_Bresistance phenotype in CoNS isolates

	Erythromycin R (n=55)				
	Clin R $cMLS_B$	Clin S D test+ve ($iMLS_B$)	Clin S D test -ve (MS_B)	Ery S + Clin S n=28	Total
Total CoNS n=83	35 (63.63%)	2 (3.6%)	18 (32.72%)	28	83

Ery=Erythromycin, Clin=Clindamycin, R=Resistant, S=Sensitive,

Table 2: Distribution of MLS_B resistant phenotypes among MR-CoNS

	Erythromycin R (n=55)				
	Clin R $cMLS_B$	Clin S D test +ve ($iMLS_B$)	Clin S D test -ve (MS_B)	Ery S + Clin S n=28	Total
MR-CoNS n=26	19 (73.07%)	0	6 (23.07%)	1	26
MS-CoNS n=57	16 (28.07%)	2 (3.5%)	12 (21.05%)	27	57
Total	35	2	18	28	83

The occurrence of $cMLS_B$ phenotype in MR-CoNS as compared to those in MS-CoNS was statistically significant ($p<0.05$).

in abscesses and dosage adjustment in renal disease is not required. It has good oral absorption and is an attractive option for treatment of outpatients and in de-escalation of intravenous therapy.[19]Hence, the low incidence of $iMLS_B$ in our set up means this useful antibiotic can be used as an alternative against erythromycin resistant strains. It is important to know the incidence of $iMLS_B$ in settings where clindamycin is empirically prescribed.[17,20]

True clindamycin susceptibility (MS_B phenotype) was observed in 18 (32.7%) of CoNS isolates. Patients with infections by such strain can be treated with clindamycin without the risk of rapid emergence of resistance during therapy.[8]

Comparing therate of different MLS_B phenotypes among the MS-CoNS and MR-CoNS, it was observed that 73.07% of MR-CoNSwere also $cMLS_B$ resistant while only 16 (28.07%) of MS-CoNS were $cMLS_B$ resistant. True clindamycin susceptibles (MS_B phenotype) were low in both the groups. This high rate of $cMLS_B$ resistance is a serious problem since all the MLS_B antibiotics are ineffective in the already limited options available for treatment of infections by MR-CoNS. A study on *Staphylococcus aureus* from Nepal report 94.7% methicillin resistant strains as $cMLS_B$ resistant phenotype.[21]Table 3 shows the incidence of different MLS_B resistant phenotypes at different places.

Table 3: Incidenceof different MLS_B resistant phenotypes from different places.

References	$cMLS_B$	$iMLS_B$	MS_B
Sakar H[22]	24.2%	20.3%	23.6%
Fonda BA[23]	38.09%	11.9%	32.14%
Juda M[10]	36%	18.7%	45.3%
Present study	63.63%	3.6%	32.7%
Among MR-CoNS			
TokaOzer T[24]	NA	29.2%	NA
N Pal[25]	51.57%	23.15%	25.26%
Present study	73.07%	0%	23.07%

The difference in the rate of occurrence of these phenotypes from different regions of the world could be due to the difference in the circulating clones[26] and types of infections.[8]

In the CoNS isolates analyzed in our study, $cMLS_B$ phenotype predominated. Therefore MLS_B antibiotics for infections with CoNS should not be prescribed without evidence of susceptibility from the susceptibility test. Although only two isolates had $iMLS_B$ resistancephenotype, theprevalence may change over time. Therefore we recommend the inclusion of D-test in the routine susceptibility test to differentiate the clindamycin sensitive strains into inducible resistant strains and truly susceptible strains. This can be valuable information to guide therapy in infections by CoNS resistant to erythromycin.

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