Prevalence of inducible Clindamycin resistance in Staphylococcal isolates in a tertiary care hospital in Odisha

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ABSTRACT

Background: The increasing prevalence of methicillin resistance among Staphylococci is an increasing problem. Vancomycin has been used increasingly to treat MRSA infections. Rapid increase in vancomycin resistance necessitates the restriction of its use, simultaneously also encourages treatment with older antimicrobial agents like clindamycin.

Aim: To study the prevalence of inducible clindamycin resistance in Staphylococcus aureus and Coagulase negative Staphylococci (CONS) isolated from clinical samples.

Method: This is a prospective study, conducted in a tertiary care hospital at Bhubaneswar, Odisha, from June 2009 to February 2010. A total of 332 Staphylococcal isolates were identified. After species identification, antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method. D-zone test was performed to detect inducible clindamycin resistance.

Result: Out of 332 Staphylococcal isolates, 194 (59%) were MRSA, 116(35%) MSSA, 14(4%) MRCONS and 8 (2%) strains were MSCONS. Sixty (18%) strains were D-zone positive i.e. of inducible MLS_B phenotype. Out of 60 $iMLS_B$ phenotype Staphylococcus aureus strains, 38(63%) were from urine followed by pus (20). All the $iMLS_B$ phenotype Staphylococcus aureus strains were sensitive to vancomycin and linezolid. All $iMLS_B$ phenotype were MRSA.

Conclusion: The double disk diffusion test (D-zone test) is a simple and reliable method, which should be performed routinely by all clinical microbiology laboratories to guide the clinicians about the $iMLS_B$ phenotype of Staphylococcus aureus to prevent misuse of antibiotics.

Key words: Staphylococcus, inducible clindamycin resistance, D-zone test

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens with ability to cause wide range of infections. Although healthy children have a small risk for serious infections, they could be common carriers of this organism and many clinical infections may develop in nasal carrier individuals.[1] Incidence of invasive infections has been rising with emergence of community- and hospital acquired methicillin resistant Staphylococcus aureus (MRSA).[2] MSRA is a notorious nosocomial pathogen prevalent in many countries. Vancomycin has been increasingly used to treat MRSA infections. Dissemination of vancomycin resistant enterococci was considered due partly to increased vancomycin use. Vancomycin intermediate Staphylococcus aureus (VISA) and Vancomycin resistant Staphylococcus aureus (VRSA) are also being reported from some parts of India.[3,4] Rapid increase in vancomycin resistance necessitates the restriction of its use, simultaneously also encourage treatment with older antimicrobial agents like clindamycin and trimethoprim-sulfamethoxazole.

Clindamycin, a lincosamide antibiotic, is active against Gram-positive organisms including Staphylococci and Streptococci, and acts by inhibiting bacterial protein synthesis. It is an alternative choice for mild to moderate MRSA infections. It has very good tissue penetration power (except central nervous system) and gets accumulated in abscesses, and no dosage adjustments are required in presence of renal disease.[5] The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics is commonly used in the treatment of Staphylococcal infections. However one important issue in clindamycin treatment is the risk of clinical failure during therapy.[1] Therapeutic failures caused by MLS_B inducible resistance are being more commonly reported. The MLS_B family of antibiotics have three different mechanism of resistance:

target site modification, enzymatic inactivation and macrolide efflux pump.[6] Low levels of erythromycin are the most effective inducer of inducible MLS_B resistance.[7]

Antimicrobial susceptibility data are important for the management of infections; however, false susceptibility results may be obtained if isolates are not tested for inducible clindamycin resistance.[8] The clinical and laboratory standard institute (CLSI) recommends testing for inducible clindamycin resistance in isolates of Staphylococci by using a D-zone test.[9] Aim of this study is to determine the prevalence of inducible clindamycin resistance in Staphylococcus aureus and coagulase negative Staphylococci (CONS) isolated from the patients in a tertiary care hospital in Odisha, India.

MATERIAL AND METHODS

The present study was conducted in a tertiary care hospital at Bhubaneswar, Odisha, from June 2009 to February 2010. A total of 332 Staphylococcal isolates were identified from various clinical samples. The species were identified using standard procedures.[10] Antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method.[11] D-zone test was performed to detect inducible clindamycin resistance. A Staphylococcal suspension equivalent of 0.5 McFarland turbidity was used to inoculate on Mueller-Hinton agar (MHA) plate, as per CLSI guidelines. Then 2 µg clindamycin and 15 µg erythromycin disk (from Hi-Media, Mumbai) were placed 15 mm apart (margin to margin).[1] Plates were analyzed after 18 hrs of incubation at 35^{0}C. Interpretation of diameters of zone of inhibition was as follows: ER-Sensitive (ER-S) 23mm, ER Intermediate sensitive 14-22mm and ER resistance 13mm; CL- Sensitive (CL-S) 21mm, CL intermediate sensitive 15-20mm and CL resistance 14mm. If the ER zone is 13mm and the CL zone is 21mm and both have a circular shape, the organism is negative for inducible resistance (D-test negative). If the ER zone is 13 mm and CL zone is 21 mm with a D-shaped zone around the CL, the organism is positive for inducible resistance (D-test positive).[12](Fig-1)

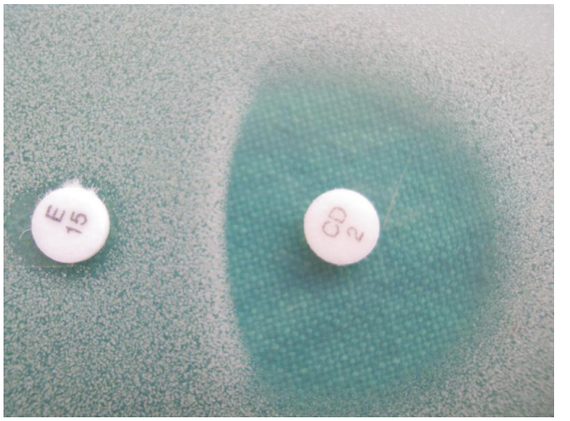

Figure 1

RESULTS

Out of 332 Staphylococcal isolates, 194 (59%) were MRSA, 116 (35%) Methicillin Sensitive Staphylococcus aureus (MSSA), 14 (4%) Methicillin-Resistant Coagulase Negative Staphylococci (MRCONS) and 8 (2%) Methicillin-Sensitive Coagulase Negative Staphylococci (MSCONS). Sixty (18%) strains were D-zone positive i.e. of inducible MLS_B ($iMLS_B$) phenotype, which were resistant to erythromycin and sensitive to clindamycin by routine Kirby-Bauer disc diffusion method. [Table-1] Out of 60 $iMLS_B$ phenotype Staphylococcus aureus strains, 38 (63%) were from urine, followed by 20 (34%) from pus. [Table-2] All the $iMLS_B$ phenotype Staphylococcus aureus strains were sensitive to vancomycin and linezolid (100%) whereas 52 (87%) strains were sensitive to teicoplanin (Teicoplanin resistance was also confirmed by E-test), and 40 (67%) strains to doxycycline. [Table-3]

DISCUSSION

The increasing frequency of Staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin (CL) therapy to treat such infections.[2] CL-resistance can develop in Staphylococcal isolates with the inducible phenotype and spontaneous constitutively resistant mutant have been selected from such isolates both in-vivo and in-vitro during CL therapy. In 1969, McGehee et al., demonstrated the

Table 1: Susceptibility to ER &CL among all Staphylococcal isolates

Phenotype	MRSA(n=194)	MSSA(n=116)	MRCONS(n=14)	MSCONS(n=8)	Total
ER-S, CL-S	16 (8%)	44 (38%)	2 (14%)	6 (75%)	68 (20%)
ER-R, CL-R ($cMLS_B$)	42 (22%)	6 (5%)	2 (14%)	2 (25%)	52 (16%)
ER-R, CL-S (MSB)(D –ve)	84 (43%)	58 (50%)	10 (72%)	0 (0%)	152 (46%)
ER-R, CL-S ($iMLS_B$)(D +ve)	52 (27%)	8 (7%)	0 (0%)	8 (2%)	60 (18%)
Total	194 (59%)	116 (35%)	14 (4%)	0 (0%)	332 (100%)

Abbreviations:

ER- Erythromycin CL- Clindamycin S- Sensitive
R- Resistant
MRSA- Methicillin Resistant Staphylococcus Aureus
MSSA- Methicillin Sensitive Staphylococcus Aureus
MRCONS- Methicillin Resistant Coagulase negative Staphylococci
MSCONS- Methicillin Sensitive Coagulase negative Staphylococci
$cMLS_B$ - Constitutive resistance $iMLS_B$ -Inducible resistance

Table 2: Isolation of Inducible MLS_B ($iMLS_B$) Staphylococcus aureus strains from different clinical samples

Specimen	Inducible MLS_B Staphylococcus aureus strains (n=60) Number & percentage
Urine	38 (63%)
Pus	20 (34%)
Blood	02 (3%)

Table 3: Antibiotic sensitivity profile of inducible MLS_B Staphylococcus aureus strains (n=60)

Antibiotics	Number of susceptible strains
Vancomycin	60 (100%)
Linezolid	60 (100%)
Teicoplanin	52 (87%)
Gentamycin	40 (67%)
Doxycycline	26 (43%)
Gatifloxacin	9(15%)
Ciprofloxacin	8 (13%)

development of CL-R in-vivo and in-vitro in ER-resistant Staphylococci.[13] Other authors have confirmed the rapid in-vitro conversion of inducible to constitutive MLS_B resistance in Staphylococci.[14] The 2004 NCCLS guidelines recommend the use of double disc diffusion test and suggest that isolates with inducible resistance phenotype should be reported as CL resistant.[15]

In our study 60 (18%) Staphylococcus aureus strains were of $iMLS_B$ phenotype. The reported rates varies across different studies: Mallick SK et al., (18.6%), Angel et al., (23.2%) and Fiebelkorn et al., (28%).[1,16,17] Among the MSSA strains, 7% were $iMLS_B$ phenotype, which was similar (4% to 15%) to reports by other researchers.[18,19] We observed the prevalence of MRSA strains to be 59%, which is somewhat similar to the results obtained by Mallick SK et al. (51.6%).[16]

CONCLUSION

MRSA has been identified as one of the clinically important multi drug resistant organism (MDRO) and therapeutic options for it are limited. In the light of restricted range of antibiotics available for the treatment of MRSA infections and known limitations of vancomycin, CL should be considered for the management of serious soft tissue infections.[20] Consequently, treatment-using CL can be omitted in patients with infections caused by

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