RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

Detection of Inducible Clindamycin Resistance in Staphlylococcous aureus isolates

KEYWORDS

*** Dr.B.Sreekanth**
Associate Professor, DM WIMS, Meppadi, Wayanad Dist, Kerala.

Dr. Gogi Suresh Dattaraya
Prof & HOD Dept of Microbiology.
DMWIMS, Meppadi, Wayanad Dist, Kerala.

ABSTRACT *Introduction :- The resistance to antimicrobial agents among staphylococci is an increasing problem. Clindamycin is considered to be one of the alternative agents in these infections. Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics.*

Materials and methods :- A total of 238 S. aureus isolates were subjected to routine antibiotic susceptibility testing including oxacillin (1 µg) and cefoxitin (30 µg) by modified Kirby Bauer disc diffusion method. Inducible resistance to clindamycin in S. aureus was tested by 'D test' as per CLSI guidelines.

Results :- Out of 238 Staphylococcus aureus isolates, 128 (53.7%) were methicillin sensitive (MSSA) and 114(47.8%) methicillin resistant (MRSA). Over all 26(18%) isolates showed constitutive resistance (MLSBc), 50(35%) inducible clindamycin resistance, 68(47%) MSB phenotype. Constitutive and inducible resistance to clindamycin were significantly higher in MRSA than MSSA (P<0.001).

Conclusion :- D-test should be included in routine antibiotic susceptibility testing for the optimum treatment of patients

Introduction
The resistance to antimicrobial agents among staphylococci is an increasing problem. Clindamycin is considered to be one of the alternative agents in these infections. Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics.

Materials and methods
A total of 238 S. aureus isolates were subjected to routine antibiotic susceptibility testing including oxacillin (1 µg) and cefoxitin (30 µg) by modified Kirby Bauer disc diffusion method. Inducible resistance to clindamycin in S. aureus was tested by 'D test' as per CLSI guidelines.

Results
Out of 238 Staphylococcus aureus isolates, 128 (53.7%) were methicillin sensitive (MSSA) and 114(47.8%) methicillin resistant (MRSA). Over all 26(18%) isolates showed constitutive resistance (MLSBc), 50(35%) inducible clindamycin resistance, 68(47%) MSB phenotype. Constitutive and inducible resistance to clindamycin were significantly higher in MRSA than MSSA (P<0.001).

Conclusion
D-test should be included in routine antibiotic susceptibility testing for the optimum treatment of patients

Introduction
Staphylococcus aureus (S. aureus) is recognized as one of the most common organisms causing nosocomial and community-acquired infections1. With the emergence and swift spread of methicillin resistant Staphylococi (MRSA) both in hospitalized patients and the community, the betalactam group has considerably lost its deterrence2. Wide spread use of macrolide-lincosamide-streptogramin (MLS) antibiotics, has led to increase in number of staphylococcal acquiring cross resistance to MLS antibiotics3. Resistance to MLS antibiotics occur either through efflux of antibiotics or target site modification4. Efflux mechanism is encoded by mrs(A) genes and confers resistance to macrolides and type B streptogramin (MSB resistance). Target site modification, mediated by erm genes leads in resistance to macrolides, lincosamides and type B streptogramin (MLSB antibiotics) 3. The expression of the MLSB phenotype can be constitutive (MLSBc) or inducible (MLSBi). While constitutive MLSB resistace (MLSBc) shows in vitro resistance to macrolides, lincosamides and type B streptogramins, strains with inducible resistance (MLSB i) demonstrate in vitro resistance to erythromycin but appear susceptible to lincosamides in the standard susceptibility testing2. Low levels of erythromycin is an inducer of the MLSBi phenotype, which forms the basis of the D-test4. It is important to determine MLSBi phenotype. Treatment of an infection by MLSBi phenotype with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. In case of MSB phenotype, Staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro and the strain do not typically become clindamycin resistant during therapy1. The present study was aimed to detect inducible clindamycin resistance (MLSBi) in methiciliin resistance staphylococci (MRSA) and methicillin sensitive staphylococci (MSSA).

Materials and Methods
The present was conducted from March 2013 to Febrauary 2014. A total of 238 S. aureus were isolated from various clinical specimens (pus, wound swab, sputum, aspirates and blood). Strains were identified using conventional bacteriological methods and their susceptibility testing was first performed by standard disk diffusion method on Muller Hinton agar(MHA) using erythromycin (15ug), clindamycin (2 µg), vancomycin (30 µg), oxacillin (1 µg), and cefoxitin (30 µg), according to clinical and laboratory standards institute (CLSI) guidelines[5]. An inhibition zone of 10 mm or less around oxacillin disc and 19 mm or less around cefoxitin disc indicates MRSA. Each erythromycin resistant clindamycin sensitive isolates were further tested for inducile clindamycin resistance with D –test, as described in the CLSI recommendations.

Isolates with MS_B resistance demonstrated circular clindamycin zone, while isolates with MLSBi phenotype, demonstrated a flattening of the clindamycin zone. Isolates resistant to both erythromycin and clindamycin confers MLSBc resistance phenotype3.

Results
Among the 239 isolates studied, 114(47.6%) were MRSA and the rest 125 (52.3%) were MSSA. of the 239 isolates, 144

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

(60.2%) were resistant to erythromycin (ERY-R). D- test was performed and found that, percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA isolates [Table 1]. Truly susceptible isolates (MS_B phenotype) were significantly higher in MSSA strains than in MRSA strains ($P < 0.001$). Falsely susceptible isolates (MLSBi phenotype) were significantly higher in MRSA strains than in MRSA strains ($P < 0.001$). However no statistically significant difference of truly resistant isolates (MLSBc phenotype) was observed between MRSA and MSSA strains.

Discussion

Clindamycin is a good alternative for the treatment of both meticillin-resistant and -susceptible staphylococcal infections. Clindamycin resistance can develop in staphylococcal isolates with the inducible phenotype, and spontaneous constitutively resistant mutants have been selected from such isolates both in vitro and in vivo during clindamycin therapy[6]. However, one of the major concerns regarding the use of clindamycin to treat staphylococcal infections is the possible presence of inducible resistance to clindamycin, and subsequent failure of clinical therapy[7]. A simple, reliable, D- test can be used to delineate inducible and constitutive clindamycin resistance in routine clinical laboratories. In our study, 33% of strains were MS_B phenotype and 45% were MLSBi Phenotype among the MRSA strains, resulting in an underestimated clindamycin resistance rate of 22% (MSLBc) instead of 67% (MLSBi and MLSBc) and similarly, 14% instead 37% among MSSA [Table 1]. Other, similar to our study, showed that percentage of inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA[1].

Conclusion

Clinical microbiology laboratories should use the simple, reliable, D-test along with routine susceptibility testing as standard practice with all erythromycin resistant staphylococci. Consequently, treatment using clindamyciny can be omitted in patients with infections caused by MLSBi strains, and therapeutic failures may thus be avoided.

Table.1: Determination of Inducible clindamycin resistance among MRSA and MSSA Isolates

	MRSA	MSSA	Total
MLSBc	17 (22)	09 (14)	26 (18)
MLSBi	35 (45)	15 (23)	50 (35)
MS_B	26 (33)	42 (63)	68 (47)
Total	78 (100)	66 (100)	144 (100)

REFERENCE

1. Kavitha P, Sunil Rao, Venkatakrishna Rao. Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Clinical Samples. J Lab Phy 2011; 3(1):25-27. | | 2. Bashir FA, Maroof PA, Zahoor D, Manzoor TA, Reyaz NA. Phenotypic Detection of Constitutive and Inducible Clindamycin Resistance in Clinical Isolates of Staphylococcus aureus and Coagulase Negative Staphylococcus on Routine Susceptibility Plate. J. Commun. Dis 42 (1):2010;19-26. | | 3. Aleksandra et al. Prevalance of inducible clindamycin resistance among community- associated staphylococcal isolates in central Serbia. Ind J Med Micro2014;32(1):49-52. | | 4. Ciraj. A M, Vinod. P, Sreejith. G, Rajani. K. Inducible clindamycin resistance among clinical isolates of staphylococci. Ind J Path and Micro 2009;52(1):49-51. | | 5. Clinical laboratory standards institute (CLSI)guidelines. performance standards for antimicrobial susceptibilty testing: Ninetieth informational supplement. CLSI documentM100-S19. clinical and laboratory standards institute. Pennsylvania: Wayne;2009.p.59. | | 6. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol 2007;56:342–345. | 7. Kumar.S. Detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci - a study from South India. Internet J Microbiol 2010 ;(9)2. |