Research Paper Medical Science

Prevalence of Inducible Clindamycin Resistance Among Clinical Isolates of Mrsa in Malwa Region of Punjab (North India)

* Dr Neerja Jindal ** Dr Seema Singh *** Dr Pragati Grover **** Dr Rubina Malhotra

* Prof.&Head, Department of Microbiology, GGS Medical college, Faridkot, Punjab(151203)

** JR-3,Department of Microbiology GGS Medical college, Faridkot, Punjab(151203)

*** JR-3,Department of Microbiology GGS Medical college, Faridkot, Punjab(151203)

**** SR,Department of Microbiology GGS Medical college, Faridkot, Punjab(151203)

ABSTRACT

Clindamycin is a useful alternative drug for the treatment of infections caused by MRSA. However, in vitro routine tests for clindamycin susceptibility usually fail to detect inducible clindamycin resulting in treatment failure. This type of resistance in clindamycin varies by different geographical regions and could be detected by D test on routine basis. Of the total of 288 Staphylococcal isolates studied, 116(40.27%) were found to be MRSA - resistant to cefoxitin (30 µgm/ disc). Of these, 54 isolates were resistant to erythromycin but sensitive to clindamycin. D test performed on these showed that it was positive in 21(18.1%) indicating inducible clindamycin resistance. In MSSA, inducible clindamycin resistance was observed in 10(5.81%). There was statistically significance difference between MRSA and MSSA isolates showing inducible clindamycin resistance (p=0.000). As inducible clindamycin resistance is not that uncommon (18.1%) in our hospital, D test should always be performed in S.aureus isolates, showing clindamycin–erythromycin discordance on disc diffusion test to avoid erroneous reporting resulting in treatment failure.

Keywords : MRSA; Clindamycin; D test; Inducible clindamycin resistance

Introduction:

Over the last decade, a considerable increase in the prevalence of MRSA (Methicillin resistant Staphylococcus aureus) has been reported from almost every region of world[1]. The infections due to MRSA are difficult to treat because of the restricted spectrum of antimicrobials of proven efficacy. Clindamycin, a macrolide-lincosamide-streptogramin B (MLS_B) antibiotic, having excellent pharmacokinetic properties is a good substitute to treat these infections[2]. However, there are reports of development of resistance to this drug too[2,3]. Macrolide (MLS_B) resistance may be due to enzyme encoded by a variety of erm genes, which may be expressed either constitutively (MLSBc phenotype) or inducibely (MLSBi phenotype). Other mechanism of resistance is mediated through active efflux pump encoded by msr A gene (MS phenotype)[3].

In vitro susceptibility test for clindamycin usually fail to detect inducible clindamycin resistance as it appears as erythromycin resistant and clindamycin sensitive. In-vivo therapy with clindamycin in such cases may result in treatment failure, thus necessitating the need to detect this resistance by D test (Double disc diffusion test)[2]. Since, the prevalence of inducible clindamycin resistance may vary in different geographical regions, different hospitals and by methicillin susceptibility[4,5], the present study was undertaken, to determine the prevalence of inducible clindamycin resistance (MLSBi) in MRSA in Malwa region of Punjab from where such studies are lacking.

Material & Methods

A total of 288 consecutive strains of Staphylococcus aureus isolated from various clinical specimens obtained in the Microbiology department during the period of two years (July 2011 to June 2013) were included in this prospective study. The isolates were identified as S.aureus by conventional microbiological methods and slide and tube coagulase test[6].

Antibiotic susceptibility testing was performed by kirby Bauer's method on Muller Hinton agar using antibiotics discs of ampicillin (10 µgm), erythromycin (15µgm), clindamycin (2 µgm), vancomycin (30 µgm), netilimicin (30 µgm), cefoxitin (30 µgm), ciprofloxacin (15 µgm), gentamycin(10µgm), linezolid (30 µgm), gatifloxacin (5 µgm) as per CLSI guidelines. An isolate showing an inhibition zone of ≤ 22mm around cefoxitin disc was identified as MRSA[7]. All the isolates which showed clindamycin-erythromycin discordant sensitivity results were further subjected to D test as per CLSI guidelines. Briefly, erythromycin (15 µgm) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µgm) disc on Muller Hinton agar, previously inoculated 0.5 Mcfarland bacterial suspension. Following overnight incubation at 37^0C, flattening of zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance. Three phenotypes were appreciated after testing and interpreted as follows[2]:-

- Constitutive (MLSBc) Phenotype- Isolates which showed resistance to both erythromycin (zone size≤13mm) and clindamycin (zone size ≤14mm).
- Inducible (MLSBi) Phenotype - Isolates resistant to erythromycin (zone size ≤ 13mm), sensitive to clindamycin (zone size ≧ 21mm) and giving D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.
- MS Phenotype – Isolates exhibiting resistance to erythromycin (zone size ≤ 13mm) and sensitivity to clindamycin (zone size ≧ 21mm) with circular zone of inhibition around clindamycin.

Results :-
Of the 288 S.aureus isolates studied, 116 (40.27%) were MRSA (methicillin resistant S.aureus) and remaining 172 (59.72%) were MSSA (methicillin sensitive S.aureus). Of the 116 MRSA, 35 (30.17%) were erythromycin sensitive while 81 (69.83%) showed resistance to erythromycin. Resistance to both erythromycin and clindamycin was observed in 27 (23.3%) indicating constitutive (MLSBc) phenotype. Rest of 54 strains which were resistant to erythromycin but sensitive to clindamycin were subjected to D test. The test was positive in 21 (18.1%) which indicated inducible (MLSBi) phenotype; in 33 (28.4%) isolates D test was negative and indicated MS phenotype "Table 1 about here'. Among MSSA isolates inducible and constitutive clindamycin resistance was 5.81% and 5.23% respectively "Table 1 about here'. There was statistically significant difference between MRSA and MSSA isolates in both the constitutive clindamycin resistance (p = 0.000) and inducible Clindamycin resistance (p = 0.002) "Table 1 about here'.

Discussion
Clindamycin is a good alternative drug in the treatment of skin, soft tissue and bone infections caused by both MRSA and MSSA because of its tolerability, cost, oral form, excellent tissue penetration and the fact that it accumulates in abscesses[8]. However, clindamycin resistance develops in Staphylococcal isolates with inducible phenotype and from such isolates, spontaneous constitutively resistant mutants arise both in vitro testing and in vivo during clindamycin therapy[8]. Since the MLSBi resistance mechanism in not recognized by using standard susceptibility testing, D test has become an imperative part of routine antimicrobial susceptibility testing for all clinical isolates of S. aureus[9].

In the present study, when the D test was performed, prevalence of inducible clindamycin resistance was 18.1% and constitutive resistance 23.3% "Table 1 about here'. Our finding of inducible clindamycin resistance (18.1%) is in concordance with the study of Deotale et al (2010)[3] and Sireesha et al (2012),[10] higher than those of Rahbar et al (2007)[11] and Ahmed (2010)[12] but lower than those of Pal N et al (2010 Jaipur)[13] and Prabhu et al (2011)[2].This difference could be because, the prevalence of inducible clindamycin may vary by geographical regions and even from hospital to hospital.

In our study, inducible clindamycin resistance was observed in both MRSA (18.1%) and MSSA (5.81%), although the difference between the two was statistically significant (p = 0.000). This is similar to the findings of Deotale et al[3] and Gadepalli et al[14]. On the Contrary, few studies have shown higher prevalence of inducible resistance in MSSA as compared to MRSA.[15,16]

Therefore, it is concluded that clindamycin could be considered for the management of both MRSA and MSSA infections but only after ruling out inducible resistance to clindamycin by doing a simple, inexpensive D test. The test could be performed in routine clinical laboratory without any specialized testing facilities. Its routine use in laboratory would enable us to guide the clinicians regarding judicious use of clindamycin, as it is not a suitable drug for D test positive infections, while it can definitely be a drug of choice in isolate showing negative D test.

Table 1- Susceptibility of Erythromycin and Clindamycin among MRSA (116) and MSSA (172) isolates.

	E-R C-R (MLSBc)	E-R C-S D+ (MLSBi)	E-R C-S D- (MS)	E-S C-S	Total no of isolates (%)
MRSA	27(23.3)[a]	21(18.1)[c]	33 (28.4)	35 (30.17)	116 (40.27)
MSSA	9 (5.23)[b]	10(5.81)[d]	25 (14.5)	128 (74.4)	172 (59.72)
Total	36 (12.55)	31 (10.7)	58 (20.13)	163 (56.6)	288

E-Erythromycin C-Clindamycin R-Resistant S-Senitive
MRSA – Methicillin resistant S.aureus
MSSA – Methicillin sensitive S.aureus
MLSBc – Constitutive clindamycin resistant phenotype
MLSBi - Inducible clindamycin resistant phenotype
MS – MS phenotype

Statistical analysis
Constitutive (MLSBc):- a & b (p = 0.000) Significant
Inducible (MLSBi) :-c & d (p = 0.002) Significant

REFERENCES

1. Boucher HW, Corey GR,(2008). Epidemiology of Methicillin resistant Staphylococcus aureus. Clin Infect Dis; 46 (5): 344-9 | | 2. Kavita Prabhu, Sunil Rao, Venkatakrishana Rao, (2011). Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J lab physicians.; 3(1): 25-27 | | 3. Deotale V, Mendiratta DK, Raut U, Narang P,(2010). Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J med Microbiol.; 28 : 124-6 | | 4. Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ,(2006). Prevalence of Inducible clindamycin resistance among community and hospital associated Staphylococcus aureus isolated. J clin Microbial; 44: 5481-4 | | 5. Angel MR, Balaji V, Prakash JA, Brahmandathan KN, Mathews MS, (2008). Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J Med Microbial;26:262-44 | | 6. Collee JG., Fraser AG., Marmion BP., Simmons A. & Barid D.,(1996). Staphylococcus: Cluster- forming Gram-positive cocci. Mackie and McCartney Practical Medical Microbiology (14thed), Vol.2. Churchill Livingstone London; 245-61 | | 7. Clinical and laboratory standards institute,(2011). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Clinical laboratory standards Institute.; vol.2 (No.1) | | 8. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I,(2007). Detection and prevalence of inducible clindamycin resistance in Staplylococci. J Med Microbiol; 56: 342-5 | | 9. Gupta V, Datta P, Ravi H, Chander J(2009). Inducible Clindamycin resistance in S.aureus: A study from north India. J Postgrad Med.; 55: 176-9 | | 10. Sireesha P, Setty CR, (2012). Detection of various types of resistance patterns and their correlation with minimal inhibitory concentration against clindamycin among methicillin resistant Staphylococcus aureus isolates. Indian J Med Microbiol; 30: 165-9 | | 11.Rahbar M, Hajia M,(2007). Inducible clindamycin resistance in Staphylococcus aureus: A cross-sectional report. Pak J Biol Sci; 10: 189-92 | | 12. Mohamed O. Ahmed. Detection of inducible clindamycin resistance (MLSBi) among MRSA from Libya, (2010). Libyan J Med.; 5:10 | | 13. Pal N, Sharma B, Sharma R, Vyas L,(2010). Detection of inducible clindamycin resistance among Staplylococcal isolate from different clinical specimens in western India. India J Postgrad Med; 56: 182-5 | | 14. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry R, (2006). Inducible clindamycin resistance in clinical isolates of S. aureus. Indian J Med Res; 123: 571-3 | | 15. Schreckenbuger PC, Ilendo E, Ristow KL, (2004). Incidence of constitutive and inducible clindamycin resistance in S. arueus and coagulase negative Staphylococci in a community and tertiary care hospital. J Clin Microbial; 42:27 | 16. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T,(2005). Potential clindamycin Resistance in clindamycin – susceptible, erythomycin – resistant S aureus: Report of a clinical failure. Antimicrob Agent Chemother; 49:1222-4 |