Int J Pharm Bio Sci 2013 July; 4(3): (B) 874 - 879

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INDUCIBLE CLINDAMYCIN RESISTANCE IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Inducible clindamycin resistance in *Staphylococcus aureus* is of great concern owing to its frequent association with false sensitivity in antibiogram and treatment failure. One hundred and two clinical isolates of Methicillin resistant *S. aureus* (MRSA) were identified by oxacillin, cefoxitin disc diffusion and oxacillin agar dilution method. Antibiotic susceptibility to erythromycin and clindamycin was detected by Kirby Bauer disc diffusion method. Isolates resistant to erythromycin were further tested by D test for detection of inducible clindamycin resistance. MRSA strains had high resistance to erythromycin (74.5%). Inducible clindamycin resistance was detected in 49.2% and 30.7% MRSA isolated from inpatients & outpatients and in 40% and 43.5% MRSA isolated during 2004-2009 and 2010-2011, respectively. Owing to high prevalence of inducible clindamycin resistance in MRSA, we recommend routine testing for inducible clindamycin resistance in laboratories.

KEYWORDS: **Methicillin resistant Staphylococcus aureus; D test; inducible clindamycin resistance; MLSBi**

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INTRODUCTION

Staphylococcus aureus is a pervasive pathogen in both hospital and community. Methicillin resistance in *Staphylococcus aureus* is of great concern owing to its frequent association with multidrug resistance, aggressive course, increased mortality & morbidity and outbreak potential in both community and hospital facilities. Methicillin resistant *S. aureus* (MRSA), especially hospital acquired MRSA (HA-MRSA) strains frequently acquire resistance to other non-betalactam drugs like quinolones, aminoglycosides and macrolides as a result of persistence in health care environment with greater antibiotic selection pressure.[1] Moreover, there are only a few anti-MRSA drugs on the pipeline of development for clinical use in future.[2] Consequently, the treatment options are narrowed down to only few antibiotics. In various health care setups, surgical infections & skin & soft tissue infections caused by MRSA are treated successfully by clindamycin.[3] However, in recent years the therapeutic use of clindamycin has been curtailed by emergence of inducible clindamycin resistance in *S. aureus*. Inducible clindamycin resistance easily escapes detection in laboratory in routine antibiotic sensitivity testing resulting in treatment failure, increased duration of hospital stay and dissemination of resistant strains. This study was designed to determine the prevalence of different phenotypes of clindamycin resistant strains of MRSA in our hospital.

METHODOLOGY

This study was carried out in a 750 bed tertiary care hospital in south India catering patients from Pondicherry and neighboring districts of Tamil Nadu after receiving Institutional ethical committee clearance. Forty MRSA isolates from clinical materials preserved from March 2004–December 2009 and 62 new isolates recovered during January, 2010 to June, 2011 from various clinical samples such as pus swabs & aspirates, blood, urine, sputum & endotracheal tube aspirate, were included in this study. Consecutive isolates of MRSA from same patient are excluded.

1. Isolation & identification of Staphylococcus aureus

All clinical samples are processed in the laboratory following standard guidelines. *S.aureus* isolates were identified by gram stain, colony morphology on blood agar, MacConkey's agar, mannitol salt agar, MeReSA selective agar (Himedia, Mumbai, India), catalase, tube coagulase and DNase test.

2. Detection of Methicillin resistant Staphylococcus aureus

Disc diffusion test using 30µg cefoxitin disc & 1µg oxacillin disc and oxacillin screen agar (containing 6 µg /ml oxacillin & 4% NaCl) tests were performed as per CLSI guideline to detect MRSA stains.[4] Antibiotic susceptibility to erythromycin and clindamycin is detected by Kirby Bauer disc diffusion method.

3. Detection of inducible clindamycin resistance

Erythromycin resistant MRSA isolates (zone of inhibition ≤13mm) were subjected to erythromycin and clindamycin disc approximation test (D test) for detection of inducible clindamycin resistance as per CLSI guidelines. A lawn culture of MRSA isolates was prepared on Mueller Hinton agar with 0.5 MacFarland colony suspension as inoculum. Erythromycin (15 µg) disc & clindamycin (2 µg) disc are placed edge to edge 15mm apart and incubated overnight at 37^{O}C. Three different phenotypes of MRSA strains can be detected.[5] Isolates with resistance to both erythromycin and clindamycin are MLSBc phenotype with constitutive clindamycin resistance. In contrast, MLSBi (inducible clindamycin resistance) phenotype strains shows sensitive zone around clindamycin disc (zone size ≥21mm)

with a flattening towards erythromycin disc (Figure 1) and MS phenotype strains are sensitive to clindamycin (zone size ≥21mm) with a circular zone without flattening towards erythromycin disc.[5]

4. Quality Control

S.aureus ATCC 25923 and ATCC 43300 were used as controls for antibiotic susceptibility test. The viability of test isolates was maintained by periodic subculture in semisolid nutrient agar..

5. Statistical analysis

All data entered in Microsoft Excel 2007 spreadsheet and statistical analysis was done in GraphPad InStat version 3.00 (San Diego, CA, USA). Chi-square test was used to compare two groups. All p values < 0.05 were considered statistically significant.

RESULTS

In the study population 102 patients, 39 were outpatient and remaining 63 were inpatients. The prevalence of erythromycin resistance in MRSA was 74.5% (n=76), of which 43 (42.1%) were D-test positive. The resistance pattern of MRSA isolates to erythromycin and clindamycin in inpatients & outpatients and in past & recent years is compared in table 1 and table 2 respectively.

Table 1
Resistance pattern of MRSA isolates to erythromycin and clindamycin in Inpatients & Outpatients (D-test)

	Erythromycin & clindamycin sensitive	**Erythromycin resistant**		
		MLSBc phenotype Clindamycin resistant & D test negative	**MLSBi phenotype** Clindamycin sensitive & D test positive	**MS phenotype** Clindamycin sensitive & D test negative
OPD (n=39)	14 (35.8%)	3 (7.7%)	12 (30.7%)	10 (25.6%)
IPD (n=63)	12 (19%)	2 (3.1%)	31 (49.2%)	18 (28.5%)
Total (n=102)	26 (25.5%)	5 (4.9%)	43 (42.1%)	28 (27.4%)

Table 2
Resistance pattern of MRSA isolates to erythromycin and clindamycin in past & recent years (D-test)

	Erythromycin & clindamycin sensitive	**Erythromycin resistant**		
		MLSBc phenotype Clindamycin resistant & D test negative	**MLSBi phenotype** Clindamycin sensitive & D test positive	**MS phenotype** Clindamycin sensitive & D test negative
2004-2009 (n=40)	9 (22.5%)	3 (7.5%)	16 (40%)	12 (30%)
2010-2011 (n=62)	17 (27.4%)	2 (3.2%)	27 (43.5%)	16 (25.8%)

Inducible clindamycin resistance was detected by positive D-test in 49.2% and 30.7% MRSA isolates from inpatients and outpatients respectively. However, the difference was not significant. Two-sided P value (Fisher's Exact Test) for MLSBi was 0.0983. (Table 1) Among MRSA isolates of 2004-2009 and 2010-2011, the proportions of D-test positive isolates having inducible clindamycin resistance were 40% and 43.5% respectively. There was no significant difference in MRSA isolates of 2004-2009 and 2010-2011 for inducible clindamycin resistance phenotype. Two-sided P value (Fisher's Exact Test) for MLSBi was 0.8378. (Table 2).

D-test for detection of inducible clindamycin resistance

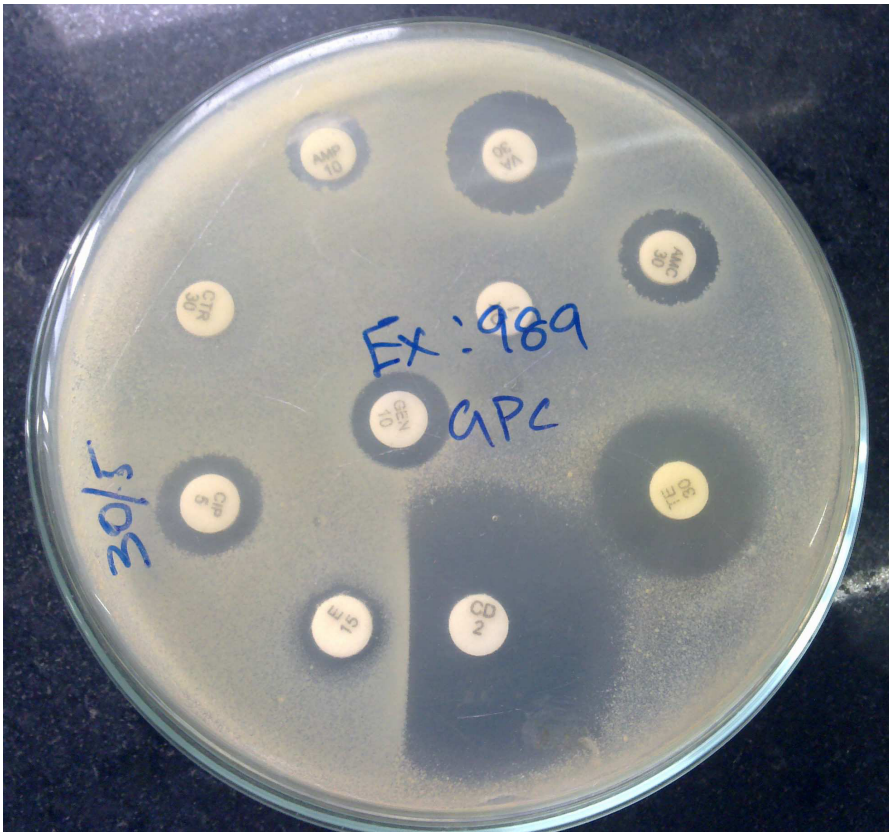

Figure 1
Zone of inhibition of clindamycin disc D-shaped flattening towards erythromycin disc

DISCUSSION

Methicillin resistant *S.aureus*[6] (MRSA), are strains of *S. aureus* which not only developed chromosomal resistance to penicillins & cephalosporins but also frequently showed resistance to wide range of antibiotics commonly used in hospitals[1]. Furthermore, old age, immunocompromised state and associated comorbid conditions are risk factor for HA-MRSA, which may preclude the use of various antibiotics. Consequently, only few treatment options are available for patients with MRSA infection. Clindamycin is a remarkable drug having good bioavailability in deep tissues and abscess cavities with lower nephrotoxicity. Clindamycin has been prescribed in skin and soft tissue infection often empirically due to better activity against both *S.aureus* and anaerobes. In recent years, the therapeutic use of clindamycin has been curtailed by emergence of clindamycin resistance in *S. aureus*. In contrast to constitutive clindamycin resistance (MLSBc) phenotype, isolates with inducible clindamycin resistance (MLSBi) is frequently not detected in conventional antibiotic susceptibility tests leading to treatment failure.[7] These MLSBi stains possess erm gene which codes for an enzyme responsible for the methylation of adenine residue in the 23s rRNA and confers resistance to streptogramin B as well as clindamycin and macrolides.[8] Efflux pumps coded by msrS genes also have been described to confer resistance to macrolides & streptogramins.[9] Although various detection methods *viz.,* disc diffusion, broth microdilution and molecular detection are in use, erythromycin & clindamycin disc approximation

test (D-test) has achieved good popularity owing to simplicity of the test. Significant proportion of MRSA strains expressing inducible clindamycin resistance has become a concern to physicians and surgeons. All erythromycin resistant strains should be tested. Depending on the result of D-test, erythromycin & clindamycin susceptibility, three phenotypes (MLSBi, MLSBc & MS) are recognized. Although CLSI recommends 15-26mm should be the distance between clindamycin & erythromycin discs, Ajantha *et al* has compared the optimum distance and was able to detect a significant number of additional MLSBi strains (5 strains, 16%) with 15mm inter disc distance than 21 mm[10]. There are reports of higher inducible clindamycin resistance in hospital acquired MRSA isolates, compared to community acquired MRSA isolates.[11] This may be attributed to frequent exposure of hospital acquired MRSA isolates to clindamycin in hospital setup. Among 102 MRSA, we identified 5 (4.9%) MLSBc, 43 (42.1%) MLSBi and 28 (27.4%) MS phenotype (table 1). Inducible resistance was more in inpatients (49.2%) compared to outpatients (30.7%). Three studies done in different set up have detected high prevalence of clindamycin resistance—27.6%, 44.4% and 38.4% respectively for inducible resistance and 7.3%. 39.7% and 15.35 respectively for constitutive resistance[12-14]. Our study results correctly corroborate with this findings. On the other hand, studies done by Ciraj *et al*, Shenoy *et al* and Pal N *et al* has reported a lesser proportion—15.65%, 16.3 and 9.38% inducible and 7.12%, 31.6% and 50% constitutive phenotype[14-16]. These results indicate geographical variation in clindamycin susceptibility owing to variations in MRSA strains, antibiotic usage and infection control measures. However, there was insignificant increase in inducible clindamycin resistance in 2010-2011 isolates compared to previous years (43.5% from 40%) (table 2).

CONCLUSION

This study highlights the crucial role of antibiotic susceptibility testing in clinical practice. Although there was no significant increase in clindamycin resistance in recent years, this study shows high prevalence of inducible clindamycin resistance among MRSA isolates. Therefore, antibiotic susceptibility testing guided antimicrobial therapy should be routine practiced and we recommend testing of all MRSA strains for inducible clindamycin resistance to avoid treatment failure.

REFERENCES

1. Pavillard R, Harvey K, Douglas D, Hewstone A, Andrew J, Collopy B, et al. Epidemic of hospital-acquired infection due to methicillin-resistant Staphylococcus aureus in major Victorian hospitals. Med J Aust. 1982;1:451-4.
2. Kaur N, Prasad R, Varma A. Antibiotic resistance among clinical isolates of Staphylococcus aureus and usefulness of antibiogram. Int J Pharm Bio Sci. 2013;4:957-64.
3. Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. Mayo Clin Proc. 1999;74:825-833.
4. CLSI. 2010. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S20. CLSI, Wayne, PA.
5. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al. Testing for Induction of Clindamycin Resistance in Erythromycin-Resistant Isolates of Staphylococcus aureus. J Clin Microbiol. 2005;43:1716-21.
6. Lucet JC, Paoletti X, Demontpion C, Degrave M, Vanjak D, Vincent C, et al. Carriage of methicillin-resistant Staphylococcus aureus in home care settings: prevalence, duration, and

transmission to household members. Arch Intern Med. 2009;169:1372-8.
7. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians. 2011;3:25-7.
8. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J Clin Microbiol. 2003;41:4740-4.
9. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clin Infect Dis. 2003;37:1257-60.
10. Ajantha GS, Kulkarni RD, Shetty J, Shubhada C, Jain P. Phenotypic detection of inducible clindamycin resistance among Staphylococcus aureus isolates by using the lower limit of recommended inter-disk distance. Indian J Pathol Microbiol. 2008;51:376-8.
11. Vasanthi R, Jeya M, Karthick MR. Inducible clindamycin resistance among community and hospital acquired isolates of Staphylococcus species. Int J Pharm Bio Sci. 2012;3(3):372-80.
12. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol. 2010;28:124-6.
13. Shrestha B, Pokhrel BM, Mohapatra TM. Phenotypic characterization of nosocomial isolates of Staphylococcus aureus with reference to MRSA. J Infect Dev Ctries. 2009;3:554-60.
14. Ciraj AM, Vinod P, Sreejith G, Rajani K. Inducible clindamycin resistance among clinical isolates of Staphylococci. Indian J Pathol Microbiol. 2009;52:49-51.
15. Shenoy MS, Bhat GK, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. Indian J Med Microbiol. 2010;28:152-4.
16. Pal N, Sharma B, Sharma R, Vyas L. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. J Postgrad Med. 2010;56:182-5.