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Research Article

Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates and Inducible Clindamycin resistance in Staphylococcus aureus: study at a Tertiary Care Hospital in KIMS, Bhubaneswar, India.

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Abstract

Aims: Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen in hospitals with hospital based outbreaks world-wide. The resistance to antimicrobial agents among Staphylococci is an increasing problem. The present study was carried out to estimate the prevalence of MRSA isolates in clinical specimens and to investigate the sensitivity pattern of those isolates against various antibiotics used for treating hospitalized patients. Attempt was also done to find out the percentage of *Staphylococcus aureus* having inducible clindamycin resistance (iMLS B) in our geographic area using D-test. We tried to ascertain the relationship between the isolates of Methicillin-resistant Staphylococcus aureus (MRSA) and inducible clindamycin resistant S*taphylococcus aureus*.Place and Duration of the Study: An ongoing study (July 2013- June 2014) in the Department of Microbiology, KIMS. **Methodology:** A total of 529 isolates of *S. aureus* were identified by standard laboratory procedures including catalase test, slide and tube coagulase tests, ß- haemolysis on blood agar and growth on mannitol salt agar. Subsequently antibiotic sensitivity pattern of S. aureus were determined by Kirby Baeur's disc diffusion method. **Conclusion:** Findings presented in this study indicated a high level of resistance to widely used therapeutic agents. An appropriate knowledge on the current antibiotic susceptibility pattern of MRSA is essential for appropriate therapeutic scenario. Total no. of samples 7371. *Staphylococcus aureus* isolated 529 (7.17%). MRSA isolated-190 (36%). Out of 74 CL sensitive MSSA. 45 (23.6%) showed D test positive, indicating inducible resistance and 29 (15.2%) showed D test negative which are truly CL sensitive. Out of 31 CL sensitive 9(2.6%) were D test positive indicating inducible CL resistance and 23(6.7%) were D test negative indicating true CL sensitivity.

Keywords: *Staphylococcus aureus;* MRSA; prevalence; antibiogram; antibiotics

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing severe morbidity and mortality at many hospitals world-wide. Once these organisms are introduced into a hospital, eradication may be difficult or impossible [1]. *S. aureus* is a leading cause of hospital acquired infection (HAI) and over the past 50 years it has acquired resistance to previously effective antimicrobials including the penicillinase resistant ones like methicillin [2]. These infections are associated with longer duration of hospital stay, greater use of health resources and higher treatment cost [3]. In recent years, the increase in the number of bacterial strains that show resistance to methicillin has become a serious clinical and epidemiological problem because this antibiotic is considered as the last option in the treatment of the staphylococci infection, and resistance to this antibiotic implies resistance to all *beta* lactam antibiotics [4]. *Staphlococcus aureus* is one of the most prevalent and clinically significant pathogen causing wide variety of infections ranging from mild skin and soft tissue infections to serious life threatening infections (5) Multidrug resistant strains of S.aureus have been reported with increasing frequency worldwide. Life-threatening sepsis, endocarditis, and

osteomyelitis caused by MRSA have also been reported. [5] Since resistance to multiple antibiotics among MRSA isolates is very common, there is a possibility of extensive outbreaks, which may be difficult to control. MRSA is now one of the commonest nosocomial pathogens, and asymptomatically colonized healthcare workers are the major sources of MRSA in the hospital environment. Early detection of MRSA and formulation of effective antibiotic policy is essential to prevent spread of MRSA in tertiary care hospitals. The emergence of MRSA has posed a serious therapeutic challenge. The Methicillin resistance requires the presence of the chromosomally localized *mecA* gene. The *mec A* gene is part of a mobile genetic element called SCCmec. *MecA* gene in MRSA is responsible for the synthesis of altered penicillin binding protein **(PBP-2a)** resulting in a loss of target affinity. *Staphylococcus aureus* continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India [6] to 50 per cent in South India. [7] The increasing prevalence of methicillin resistance among *Staphylococci* is an increasing problem. [8] The drug of choice for MRSA is Vancomycin but increasing prevalence of MRSA has led to the renewed interest in the usage of Macrolide Lincosamide Streptogramin B (MLS_B) antibiotics, to treat *S.aureus* infections, with Clindamycin due to its excellent pharmacokinetic properties. . [9,10] The resistance to macrolide can be mediated by *msr A* gene coding for efflux mechanism or via *erm* gene encoding for enzymes that confer inducible or constitutive resistance to macrolide, lincosamide and Type B streptogramin. [11] This resistance mechanism can be constitutive, where rRNA methylase is always produced ($cMLS_B$) or can be inducible where methylase is produced only in the presence of an inducing agent ($iMLS_B$). ERY is an effective inducer whereas CLI is a weak inducer. *In vitro Staphylococcus aureus* isolates with constitutive resistance are resistant to both ERY and CLI whereas those with inducible resistance are resistant to ERY and appear sensitive to CLI ($iMLS_B$).[12] If clindamycin is used for treatment of such an isolate ($iMLS_B$), selection for constitutive *erm* mutants occurs which may lead to clinical failure. Thus necessitating the need to detect such resistance by simple D test on routine basis.

Aims and Objectives

The emergence of MRSA has posed a serious therapeutic challenge.

Our aim is to study

The prevalence and antibiotic susceptibility pattern of MRSA isolates.

To detect inducible clindamycin resistance by D test from the total *Staphylococcus aureus* isolates which is of paramount importance from the epidemiological point of view.

Materials and Methods

The ongoing study (July 2013- June2014) in the Department of Microbiology, KIMS includes a total of529 isolates of *S. aureus*. These strains were obtained from various clinical samples like pus, sputum, urine, blood, and body fluids from the inpatients of our hospital. The specimens were cultured on blood agar and Mac Conkey agar plates and incubated aerobically at 37°C for 48 hours. The isolates were identified using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar. A suspension of each *S. aureus* isolate was prepared to a 0.5 McFarland standard and plated on Mueller Hinton agar.

Antibiotic sensitivity testing was performed for the following antibiotics: These antibiotic discs were obtained from Hi- Media, Mumbai. Antibiotic sensitivity testing was performed by Kirby–Bauer's disc diffusion method for the following antibiotics like Amikacin (30 µgm), ciprofloxacin (5 µgm), clindamycin (2 µgm), gentamicin (10 µgm), erythromycin (15 µmg), netilmycin (30 µgm), penicillin (10 units), Linezolid and vancomycin (30 µmg), S. *aureus* ATCC 25923 was used as a standard control.

Cefoxitin disk screen test

By definition, all methicillin-resistant S. aureus (MRSA) isolates carry the mecA gene, which confers resistance to all beta-lactam antibiotics, including cephalosporins and carbapenems. Apart from using molecular methods to detect the mecA gene directly,

the most accurate phenotypic test for the presence of the mecA gene in *S. aureus* is the cefoxitin disk diffusion test. Cefoxitin is used because it is a more potent inducer of mecA expression than other agents such as oxacillin and the test results are relatively easy to interpret. The test involves incubating a lawn of the test isolate on Mueller Hinton agar, 2% sodium chloride under standardized conditions with a 30 mcg cefoxitin disk. According to the Clinical and Laboratory Standards Institute (CLSI), a zone of growth inhibition around the cefoxitin disk of ≥22 mm rules out MRSA; a zone size <22 mm indicates that the mecA gene is present and the isolate should be reported as MRSA [13]

Clinical and Laboratory Standards Institute (CLSI) recommends the double disk diffusion test (D-test) to detect inducible clindamycin resistance. The erythromycin (15 µgm) disc was placed at a distance of 15 mm (edge-to-edge) from clindamycin (2 µgm) disc on a Mueller–Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D-shaped) around clindamycin in the area between the two discs shows inducible clindamycin resistance. [14].

Results

Total no. of samples processed were 7371 out of which *Staphylococcus aureus* isolated were 529 (7.17%) and MRSA were 190(36%). Out of 190 MRSA, 84 (44.2%) were both CL and ERY sensitive and 32(16.84%) were constitutively resistant to CL. Out of 74 CL sensitive MRSA, 45(23.6%) showed D test positive, indicating inducible resistance and 29 (15.2%) showed D test negative which are truly CL sensitive. Out of 339 MSSA, 286(84.3%) were both CL and ERY sensitive and 21(6.1%) were constitutively resistant to CL. Out of 31 CL sensitive MSSA 9 (2.6%) were D test positive indicating inducible CL resistance and 23 (6.7%) were D test negative indicating true CL sensitivity. So, both constitutive and inducible CL resistance is more in MRSA than MSSA .Out of the total isolates showing inducible clindamycin resistance [54/529 (10.2%)], 45/190(23.6%) were MRSA and 9/339 (2.65%) were MSSA.

Maximum no. of samples included in our study were urine (5151) followed by pus (1063) and blood (554). *Staph. aureus* were isolated maximum from pus sample 275 (51.98%) followed by urine 118(22.3%). MRSA were also isolated maximum from pus sample 90 (47.3%). (Table-1)

Table -1

ISOLATION OF MRSA FROM DIFFERENT CLINICAL SAMPLES

Type of sample	No. of sample	No. of staph .aureus isolates	MRSA
Pus	1063	275(51.98%)	**90(47.3%)**
Blood	554	82(15.5%)	23(12.10%)
Catheter tip	188	30(5.67%)	10(5.2%)
Sputum	415	24(4.5%)	6(3.10%)
Urine	5151	118(22.30%)	61(32.10%)
Total	**7371**	**529(7.17%)**	**190 (36%)**

Table. 2

Susceptibility to Erythromycin and Clindamycin among all *S.aureus* isolates

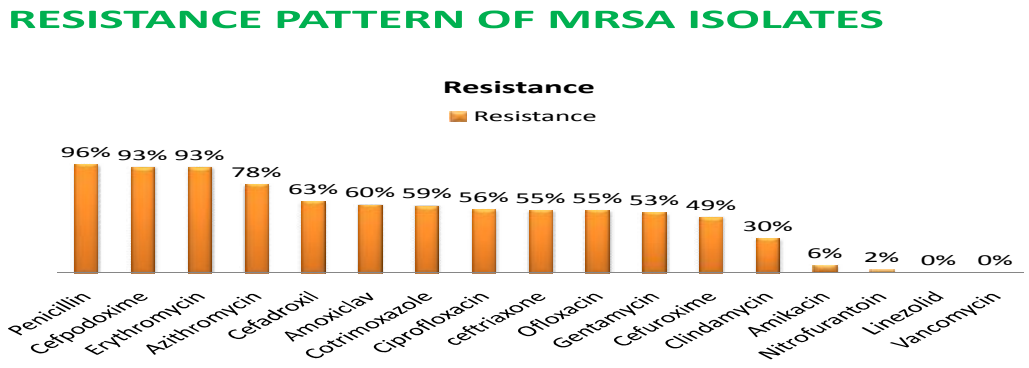
Susceptibility Pattern(Phenotype)	Number of Isolates	Percentage
ERY- S , CL-S	370	69.9%
ERY- R , CL-R (Constitutive MLS_B)	53	10.01%
ERY- R , CL-S (D-Test positive, $iMLS_B$)	54	10.20%
ERY- R , CL-S (D-Test negative, MS)	52	9.82%
TOTAL	529	100

ERY- Erythromycin, CL- Clindamycin, S- Sensitive, R- Resistant,
Constitutive MLS_B-C ERY- R , CL-S (D-Test negative, MS)
onstitutive MLS_B phenotype, $iMLS_B$ –inducible $iMLS_B$ phenotype
MS-MS Phenotype

Out of 529 *Staph. aureus* isolates, 370 (69.9%) were sensitive to both Erythromycin and Clindamycin and 53 (10.01%) were constitutively resistant. D test was positive among 54 (10.2%) S*taph. aureus* isolates and negative in 52 (9.82%) clindamycin sensitive isolates.

Maximum resistance of MRSA isolates were seen for Penicillin, Erythromycin, Cefpodoxime and Azithromycin . 100% sensitivity was seen for Linezolid and Vancomycin (Fig.1)

Fig: 1



Discussion

There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents. [15]. In our study most of MRSA isolates are resistant to Penicillin(96%), erythromycin(93%), cefpodoxime (93%), cefadroxyl (63%), amoxyclav (60%), cotrimazole (59%), Ciprofloxacin (56%) ceftriaxone (55%) and cefuroxime (49%) but these MRSA are sensitive to clindamycin (70%) and Amikacin (94%), Nitrofurantoin (98%), and 100% sensitive to linezolid, and Vancomycin. whereas Kaur et al reported isolates of MRSA showed 100% resistance to penicillin, oxacillin, ciprofloxacin and levofloxacin followed by trimethoprim-sulfamethoxazole (91.3%), erythromycin (47.8%), gentamicin (43.5%), moxifloxacin (42.9%), Less resistance was observed against tetracycline (30.4%) rifampicin (13.6%) and

clindamycin (4.8%). However, none of theMRSA isolates were found to be resistant to vancomycin, linezolid, nitrofurantoin andquinpristin/ daflopristin.[16].

Most common reason for multi drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status. There is a difference between antibiogram of MRSA and MSSA isolates and routine testing of methicillin resistance should be done using cefoxitin disc which at present is the most sensitive method .The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital showed a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008 [17].

In our study the isolation of MRSA from various clinical samples is 36%. Anupurba et al reported prevalence of 54.85% of MRSA in various clinical samples. [18] Anila A. Mathew has also reported a prevalence rate of MRSA of about 34% in clinical specimens. [19] In our study maximum number of MRSA were reported from pus samples (47.3%) which is same as the findings of Tiwari et al (17) and Anupurba et al [18]. However study carried out by Mehta et al reported 33% isolation of MRSA from pus and wound swabs [20]. Qureshi from Pakistan also reported a high isolation rate of up to 83% of MRSA from pus. [21].

In Our study out of 190 MRSA, 84 (44.2%) were both CL and ERY sensitive and 32 (16.84%) were constitutively resistant to CL. Out of 74 CL sensitive MRSA, 45 (23.6%) showed D test positive, indicating inducible resistance and 29 (15.2%) showed D test negative which are truly CL sensitive. Out of 339 MSSA, 286 (84.3%) were both CL and ERY sensitive and 21 (6.1%) were constitutively resistant to CL. Out of 31 CL sensitive 9 (2.6%) were D test positive indicating inducible CL resistance and 23 (6.7%) were D test negative indicating true CL sensitivity. So, both constitutive and inducible CL resistance is more in MRSA than MSSA he finding which is closely similar to the study carried by Deotale V (22) et al and Prabhu K et [al (23).] It is kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending upon the antimicrobial susceptibility results. Further, by using clindamycin, use of vancomycin can be avoided. However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug. In such cases, vancomycin and linezolid are the drugs which are considered for therapy. There are reports of decreased vancomycin susceptibility amongst MRSA i.e. VISA (vancomycin-intermediate *Staphylococcus aureus)* and VRSA (vancomycin-resistant *Staphylococcus aureus*). In our study we did not find any isolate showing resistance to vancomycin and linezolid. Currently, VRSA is not widespread, but it could well be the next "superbug". [24].

Conclusion

The periodic evaluation of rates for MRSA infection is crucial to both infection control monitoring and decisions regarding empirical therapy. A common method of documenting and monitoring MRSA rates is the antibiogram that reports periodically the rate of antimicrobial susceptibility for each bacterial organism and antibiotic. Generally, an antibiogram is a cumulative profile of antimicrobial susceptibility results for a given time period [25]. When properly prepared, antibiograms are important sources of information for healthcare providers. Multi-drug resistant organisms are harder to treat, poor clinical outcome, longer hospital stay, increased risk of transmission of infection to new patients, increased cost.

The percentage of isolation of MRSA from various clinical samples is 35.91%. 45 (23.6%) from 190 MRSA isolates showed D test positive indicating inducible clindamycin resistance. Linezolid and Vancomycin showed 100% susceptibility to MRSA. Clindamycin should be kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending on the antimicrobial susceptibility results. However, expression of inducible resistance to clindamycin could limit the effectiveness of the drug. The study showed a high level of MRSA in our country. There is a need to study epidemiology of such infections. Robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.

References

1. Boyce JM. Methicillin- resistant *Staphylococcus aureus.* N Engl J Med. 1989;4:901-913.
2. Duckworth GJ. Diagnosis and management of methicillin resistant *Staphylococcus aureus* infection. Br Med J. 1993;307:1049-52.
3. Engemann JJ, carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with surgical site infections. Clin Infect Dis. 2003;36:1592-98
4. Velasco D, Cartelle M, Becceiro, et al. Evaluation of different methods for detecting methicillin(oxacillin) resistance in *Staphylococcus aureus*. J Antimicrob Chemother. 2005;55:379-82.
5. Lina, G., Quaglia, A., Reverdy, Leclercq, R., Vandenesch, and Etienne. Distributon of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother*. 1999; 43: 1062–1066
6. Lowy FD.Staphylococcus aureus infections.N Engl J Med 1998;339:520-32. Patel AK, Patel KK, Patel KR, Shah S, Dileep P. Time trends in the epidemiology of microbialinfections at a tertiary care center in west India over last 5 years. J AssocPhysicians India.2010;58(Suppl):37–40. [PubMed: 21563612]
7. Gopalakrishnan R, Sureshkumar D. Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India. 2010;58(Suppl):25–31. [PubMed: 21563610]
8. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol. 2007;56:342–5. [PubMed: 17314364]
9. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis. 2005;58:104–6. [PubMed: 15858290]
10. 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. [PubMed: 20404457]
11. Laclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of resistance elements and their clinical implications. Clin Infect Dis 2002;34:482-92.
12. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother 2001;48:315-6..
13. Rasheed M, Ahmed Z: Phenotypic methods of greater accuracy to detect the mecA gene product for the recognition of MRSA in resource constraint settings. *Asian Pacific J Trop Med* 2010, 3:741-744
14. CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-firstinformational supplement. CLSI document M100-S21.Wayne, PA: Clinical andLaboratory Standards Institute. 2011;30-1 and 15
15. Mulla S, Patel M, Shah L, Vaghela G. Study of antibiotic sensitivity pattern of methicillin-resistant Staphylococcus aureus. Indian J Critical Care Medicine. 2007;11(2):99-101.
16. Kaur N, Prasad R and Varma A.Prevalence and Antibiotic Susceptibility Patternof Methicillin Resistant *Staphylococcus aureus*in Tertiary Care Hospitals,*British Biotechnology Journal4(3): 2014*
17. Tiwari HK, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance 2008;1:57
18. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM (2003): Prevalence of MRSA at a tertiary care Referral hospital in East Uttar Pradesh, Indian Journal of Medical Microbiology 21, 49-51.
19. Anila Mathews A, Marina Thomas B, Appalaraju J, Jaylakshmi (2010): Evaluation and comparison of test to detect methicillin resistant S.aureus. Indian Journal of Pathology and Microbiology. 53, 79-82
20. Mehta AP, Rodrigues C, Sheth K, Jani S, Hakimiyan A, Fazalbhoy N (1998): Control of methicillin resistant Staphylococcus aureus in a tertiary care Centre–A five–year study. Journal of Medical Microbiology 16, 31–34
21. Qureshi AH, Rafi S, Qureshi SM, Ali AM (2004): Thecurrent susceptibility patterns of methicillin resistant *Staphylococcus aureus to conventional anti Staphylococcus* antimicrobials at Rawalpindi. Pakistan Journal of Medical Science 20, 361–364

22. 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.
23. Kavitha Prabhu, Sunil Rao, and Venkatakrishna Rao.Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolated from Clinical Samples. J Lab Physicians. 2011 Jan-Jun; 3(1): 25–27.
24. Gemell CG, Edwards DI, Faise AP, Gould FK, Ridgway GL and Warren RE. Guidelines for the prophylaxis and treatment of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections in UK. J Antimicrob Chemother 2006;57:589-608.
25. Lamp K. Antibiograms. Pharm Pract Manag *Q.* 1996;16:52-6.