Original Article

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Microbiology Section

Prevalence of Methicillin, Vancomycin and Multidrug Resistance among *Staphylococcus aureus*

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ABSTRACT

Background and Objectives: The treatment of *Staphylococcus aureus* infections is becoming increasingly more complicated due to the emergence of various types of antibiotic resistance. The present study was undertaken to know the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate and vancomycin resistant *Staphylococcus aureus* (VISA and VRSA respectively) and multidrug resistant MRSA and to evaluate the phenotypic detection methods.

Materials and Methods: A total of 250 non-duplicate isolates of *Staphylococcus aureus* which were isolated from various clinical samples were tested for methicillin resistance by using the oxacillin disc diffusion test (1µg), the cefoxitin disc diffusion test (30 µg) and the oxacillin agar screen method (Muller Hinton agar (MHA) with 4% NaCl+6 µg/ml oxacillin). Vancomycin screen agar (MHA containing 5 µg/ml vancomycin) was used for screening the VISA/VRSA isolates. The MIC of vancomycin was determined by using the agar dilution method and the E-test. The antibiogram of the isolates to other antibiotics was studied by the Kirby-Bauer disc diffusion method.

Results: 80 (32%) isolates were found to be methicillin resistant by the cefoxitin disc diffusion method, 78 (31.2%) were found to be methicillin resistant by the oxacillin agar screen test and 77 (30.8%) were found to be methicillin resistant by the oxacillin disc diffusion method. No VISA and VRSA isolate was detected by using the vancomycin screen agar test, agar dilution and the E-test. The vancomycin screen agar showed 100% specificity and 100% negative predictive value. Sixty seven (83.8%) of the 80 MRSA isolates and 26.8 % of the total 250 Staphylococcus aureus isolates tested were found to be multidrug resistant MRSA.

Conclusions: Where the facilities are limited, the cefoxitin disc diffusion test and the vancomycin screen agar test can be used for screening the MRSA and the VRSA isolates respectively. With the revised CLSI guidelines, the screening method for VISA with an MIC of 4µg/ml needs to be evaluated with further more studies.

Key Words: Agar dilution, Cefoxitin disc diffusion, E-test, Methicillin resistant *Staphylococcus aureus*, Multidrug resistance, Vancomycin intermediate/resistant *Staphylococcus aureus*, Vancomycin screen agar

INTRODUCTION

Staphylococcus aureus (S.aureus) is one of the major causes of community and hospital acquired infections, leading to high morbidity and mortality [1]. The treatment of the *S.aureus* infections has become problematic because of the emergence of resistance to methicillin, vancomycin and other antibiotics [2,3]. The determination of the anti-microbial susceptibility is crucial for an optimal therapy, for epidemiological purposes and for infection control measures [3,4]. The routinely used methods cannot accurately detect the methicillin and the vancomycin resistance [5]. The present study was done to know the prevalence of methicillin and vancomycin resistance among *S.aureus*, to evaluate the phenotypic screening methods and to know the antibiogram of the isolates to the commonly used antibiotics.

MATERIALS AND METHODS

This study included a total of 250 non-duplicate *S.aureus* which were isolated from various clinical specimens from January 2010 to October 2010. All the clinical specimens were first inoculated onto blood agar and MacConkey agar plates (Hi Media Mumbai India) and these were incubated at 37^{0}C for 24-48 hours [6]. The identification of *S.aureus* was done by standard methods [5]. The screening for methicillin resistance was done by the oxacillin disc diffusion method (1µg) and by the cefoxitin disc diffusion method (30µg) and by inoculating the S.aureus onto oxacillin screen agar (Muller Hinton agar (MHA) with 4% NaCl + 6µg/ml oxacillin), (Hi Media Mumbai India) [2,7,8]. *S.aureus* ATCC 25923 was used as the control stain [9]. Screening for the vancomycin intermediate and the vancomycin resistant *S.aureus* (VISA and VRSA respectively) was carried out by using vancomycin screen agar (MHA with 5µg/ml vancomycin, Hi Media Mumbai India), its MIC was determined by the agar dilution method and it was rechecked by the E-test (Hi Media Mumbai India) [7]. All the plates were incubated at 35^0 for 24-48 hours [1,6]. *S.aureus* ATCC strains 29213 and 43300 and *E.faecalis* ATCC 51299 were used as the control strains [10]. The antibiotic susceptibility tests for the following antibiotics was carried out by the Kirby-Bauer disc diffusion method [7]; Penicillin (10U), erythromycin (15µg), tetracycline (30µg), gentamicin (10µg), amoxicillin-clavulanic acid (30 µg), clindamycin (2 µg), ciprofloxacin (15 µg), cotrimoxazole (1.25/23.75 µg), linezolid (30 µg), rifampicin (5 µg) amikacin (30 µg) and azithromycin (30 µg). This study was approved by the ethical committee of the institution.

RESULTS

Out of the 250 *S.aureus* isolates, 80 (32%) were found to be methicillin resistant by the cefoxitin disc diffusion method, 78(31.2%) were found to be methicillin resistant by the oxacillin screen agar test and 77(30.8%) were found to be methicillin resistant by the

Methods	*MRSA No (%)	**MSSA No (%)	Total No (%)
Cefoxitin disc diffusion	80 (32)	170 (68)	250 (100)
Oxacillin screen agar	78 (31.2)	172 (68.8)	250 (100)
Oxacillin disc diffusion	77 (30.8)	173 (69.2)	250 (100)

[Table/Fig-1]: Phenotypic detection methods for methicillin resistant *Staphylococcus aureus*

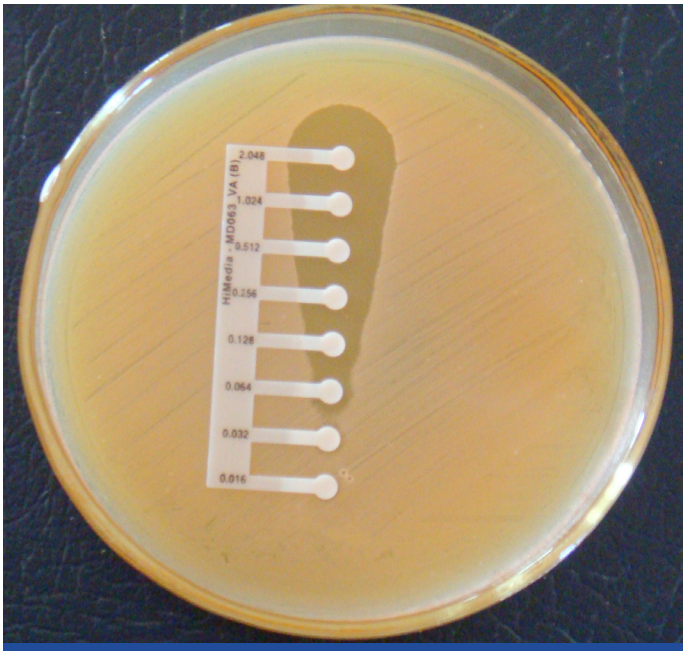
* MRSA-Methicillin resistant *Staphylococcus aureus*
**MSSA-Methicillin susceptible *Staphylococcus aureus*

Methods	*VISA	†VRSA	‡VSSA	Total
Vancomycin screen agar	Nil	Nil	250	250
Vancomycin agar dilution	Nil	Nil	250	250
Vancomycin E-test	Nil	Nil	250	250

[Table/Fig-2]: Phenotypic detection methods for vancomycin resistance in *S.aureus*

*VISA-Vancomycin intermediate *Staphylococcus aureus*
†VRSA-Vancomycin resistant *Staphylococcus aureus*
‡VSSA- Vancomycin susceptible *Staphylococcus aureus*

[Table/Fig-3]: Vancomycin MIC detection by agar dilution method



[Table/Fig-4]: Vancomycin MIC detection by E-test

Antibiotics	S.aureus (N-250)	*MRSA (N-80)	**MDR-MRSA (N-67)
Penicillin	85.6%	82.14%	92.54%
Gentamicin	33.6%	30.95%	35.82%
Amikacin	25.6%	23.80%	31.25%
Ciprofloxacin	60%	54.76%	65.67%
Erythromycin	56%	57.4%	67.16%
Clindamycin	14%	22.62%	28.36%
Tetracycline	28%	47.61%	56.72%
Cotrimoxazole	68.8%	67.86%	79.10%
Amoxicillin-Clavulanic acid	42.8%	45.24%	55.22%
Rifampicin	12.8%	27.38%	34.33%
Linezolid	2.8%	3.57%	4.48%
Azithromycin	32.8%	46.43%	52.24%

[Table/Fig-5]: Antibiotic resistance profile of S.aureus, MRSA and multidrug resistant MRSA

*MRSA- Methicillin resistant *Staphylococcus aureus*; **MDR- Multidrug resistant.

oxacillin disc diffusion method [Table/Fig-1]. No VISA and VRSA were found among the 250 *S.aureus* isolates by all three methods i.e., the vancomycin screen agar test, the vancomycin agar dilution method and by the E-test [Tables/Figs 2, 3 and 4]. The vancomycin screen agar showed 100% specificity and 100% negative predictive value. The antibiotic resistance profile of the isolates to other antibiotics is represented in [Table/Fig-5].

DISCUSSION

Staphylococci are the commonest of all the clinical isolates which are responsible for several suppurative types of infections and they are capable of acquiring and using one or more of the resistance mechanisms [2,11]. Due to the widespread occurrence of MRSA, the empiric therapy for MRSA was changed to vancomycin. This has lead to the emergence of vancomycin intermediate and vancomycin resistant *S.aureus* [3]. Because only limited therapeutic alternatives are available presently to treat the MRSA and the VRSA isolates, the detection of methicillin and vancomycin resistance should be done in the clinical microbiology laboratory with meticulous care, keeping in mind the sensitivity and the specificity of the methods which are used [3]. In the present study, 80 (32%) isolates were found to be MRSA [Table/Fig-1]. The prevalence of the *S.aureus* infections vary from place to place and so also the resistance pattern which depends on the local antibiotic policy, the infection control activities, the time of the study, the number of cases which are studied and the biological characteristics of the *S.aureus* strains [12,13]. One of the limitations of the present study was that, the detection of mecA or PBP 2a which is considered as the gold standard for detecting the MRSA strains, was not done because of technical and economic constrains [14]. Among the screening methods which were used for MRSA, cefoxitin disc diffusion was preferred over oxacillin disc diffusion and the oxacillin screen agar test because cefoxitin was a potent inducer of the mecA gene, it gave clearer end points, it was less affected by the hyper production of penicillinases, it required no special medium or incubation temperature as was required when the testing was done with oxacillin and it could be done routinely in the same antibiotic sensitivity plate [2,12,14,15]. Even with a 32% MRSA prevalence rate, no isolate with a reduced susceptibility to vancomycin was found in the present study [Table/Fig-2]. This may be due to the fact

that the community acquired MRSA (CA-MRSA) unlike the hospital acquired MRSA (HA-MRSA) are known to be sensitive to drugs other than vancomycin [16]. Because of its high cost, vancomycin may not be in use in the peripheral rural setups, thus decreasing the selection pressure for vancomycin resistance. As per the new guidelines, CLSI has suggested further studies to define the level of sensitivity of the methods for VISA, for which the MICs are 4 µg/ ml [10]. In the present study, the vancomycin screen agar test (MHA with 5µg /ml of vancomycin) was evaluated. No VISA or VRSA isolate was found in the present study and this was confirmed by the vancomycin agar dilution method [Table/Fig-3] and the E-test [Table/Fig-4]. The vancomycin screen agar test showed cent percent specificity and cent percent negative predictive value. With the revised CLSI guide lines for VISA, if only BHIA with 6 µg /ml of vancomycin is used, a fraction of the VISA isolates with an MIC of 4 µg/ml may be missed. MHA was used in the present study because MHA was widely available and it was well standardized, it readily supported the growth of *Staphylococci*, it was commercially available and it could be easily prepared in house [17]. The burden of multidrug resistant MRSA is increasing over time [18]. The reports of recent studies are implicating the gut as an important reservoir of multidrug resistant *S.aureus* [19]. In the present study, 67(83.8%) of the MRSA isolates were found to be multidrug resistant. [Table/Fig-5] The increased prevalence of MDR-MRSA is due to a lack of sufficient knowledge on the danger of the wrong use of antibiotics, high proximity to a large number of unlicensed drug vendors, high poverty among the people which hinders them from completing the dosage regimen of the antibiotics, widespread and sometimes, the inappropriate use of broad spectrum antibiotics in the medical and the veterinary practice, antibiotic prophylaxis, high number of immune compromised patients, the increased use of invasive procedures and devices and inadequate infection control measures [19,20].

CONCLUSION

Vancomycin resistance has been perceived as a fearsome threat to the already challenging therapy of MRSA and MDR-MRSA [6,18]. The clinical microbiology laboratories must ensure that they are using detection methods with good sensitivity and specificity. In the rural hospitals, where the facilities are limited, the cefoxitin disc diffusion test and the vancomycin screen agar test can be used as reliable screening methods for the MRSA and the VRSA strains respectively. More studies should be done for finding the accurate screening method for VISA. The emergence and the dissemination of resistance can be controlled by a heightened awareness of the issues, by encouraging proper personal hygiene, provision of adequate effective sewage disposal systems to prevent dissemination of the multidrug resistant bacteria from the gut, surveillance of the local bacterial population, early intervention, rigorous cross infection control measures and by the judicious use of current antimicrobial agents based on the susceptibility data [19,20,21].

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REFERENCES

[1] Bhateja P, Mathur T, Pandya M, Fatma T, Rattan A. Detection of vancomycin resistant *Staphylococcus aureus*: A comparative study of three different phenotypic screening methods. *Indian J Med Microbiol*. 2005; 23(1): 52-55.
[2] Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistant *Staphylococcus aureus*. *Indian J Pathol Microbiol*. 2010; 53(1): 79-82.
[3] Saderi H, Owlia P, Shahrbanooie R. Vancomycin resistance among the clinical isolates of *Staphylococcus aureus*. *Arch Iran Med*. 2005; 8(2):100-03.
[4] Perez LRR, Caierao J, Antunes ALS, d Azevedo PA. Use of the D test method to detect inducible clindamycin resistance in coagulase negative *Staphylococci*. *Braz J Infect Dis*. 2007; 11(2): 186-88.
[5] Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al, editors. *Koneman's Colour Atlas and Text book of Diagnostic Microbiology*, 6th edition. Philadelphia: Lippincott Williams and Wilkins; 1997.
[6] Tiwari HK, Sen MR. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) at a tertiary care hospital in the northern part of India. *BMC Infect Dis*. 2006; 6:156.
[7] Clinical and Laboratory Standards Institute/NCCLS. Performance Standards for Antimicrobial Susceptibility Testing Sixteenth Informational Supplement M100-S16, Wayne, Pa: CLSI; 2006.
[8] Swenson JM, Tenover FC, Cefoxitin Disc Study Group. Results of disk diffusion testing with cefoxitin correlated with the presence of mecA in the *Staphylococcus spp*. *J Clin Microbiol*. 2005; 43(8):3818-23.
[9] Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of the polymerase chain reaction and conventional methods in the detection of methicillin-resistant *Staphylococcus aureus*. *J Infect Dev Ctries*. 2008; 2(1): 46-50.
[10] Centers for Disease Control and Prevention. Laboratory Detection of Vancomycin-Intermediate/ Resistant *Staphylococcus aureus* (VISA/ VRSA). Available at http:// www.cdc.gov/ncidod/dhqp/ar_visavrsa_ labFAQ.html (Accessed on July 25, 2011).
[11] Forbes BA, Sahn DF, Weissfeld AS. Editors. *Bailey and Scott's Diagnostic Microbiology*, 12th edition. Missouri: Mosby Elsevier ; 2007.
[12] Mallick SK, Basak S. MRSA - too many hurdles to overcome; a study from central India. *Trop Doct*. 2010; 40: 108-10.
[13] Asghar AH, Momenah AM. Methicillin resistance among the *Staphylococcus aureus* isolates from Saudi hospitals. Med Princ. 2006; 15: 52-55.
[14] Rahbar M, Safadel N. Evaluation of the cefoxitin disc diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. *Iranian Journal of Pathology*. 2006;1(4): 145-48.
[15] Centers for Disease Control and Prevention. Laboratory Detection of Oxacillin/Methicillin-resistant *Staphylococcus aureus*. Available at http://www.cdc.gov/mrsa/lab/lab-detection.html Accessed (June 08, 2011).
[16] Fasih N, Irfan S, Zafar A, Khan E, Hasan R. Inducible clindamycin resistance which was caused due to the expression of the erm genes in *Staphylococcus aureus*: Report from a tertiary care hospital in Karachi, Pakistan. *J Pak Med Assoc*. 2010; 60:750-53.
[17] Hubert SK, Mohammed JM, Fridkin SK, Gaynes RP, McGowan JE Jr, Tenover FC. Glycopeptide- intermediate *Staphylococcus aureus*: Evaluation of a novel screening method and the results of a survey of selected U.S. hospitals. *J Clin Microbiol*. 1999; 37(11):3590-93.
[18] Tiwari HS, Sapkota D, Sen MR. The high prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. *Infection and Drug Resistance*. 2008; 1: 57-61.
[19] Onanuga A, Temedie TC. Multidrug-resistant, intestinal *Staphylococcus aureus* in self medicated, healthy adults in Amassoma, south-south, Nigeria. *J Health Popul Nutr.* 2011 Oct; 29(5):446-53.
[20] Carbon C. MRSA and MRSE: is there an answer? *Clin Microbiol Infect*. 2000; 6 suppl 2:17-22.
[21] Srinivasan A, Dick JD, Perl TM. Vancomycin resistance in *Staphylococci*. *Clin Microbiol Rev*. 2002; 15(3):430-38.

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