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ORIGINAL ARTICLE

Phenotypic Detection and Rate of Nasal Carriage of Heterotypic Borderline Oxacillin Resistant *Staphylococcus aureus* In Pre-clinical Medical Students from Malaysia

Santhosh D V *,Shobha K L **,Bairy I *,Gowrish R ****,D'Souza J *******

ABSTRACT

Background: We investigated the rate of nasal colonization of heterogeneous *Staphylococcus aureus* (Methicillin Resistant S. aureus (MRSA), borderline oxacillin resistant *S. aureus* (BORSA), as well as hetero-resistant patterns exhibited by *S.aureus*, in 157 pre-clinical medical students from Melaka Manipal Medical College, Manipal University, in order to determine the carrier profile among the student community from Malaysia before they entered the clinics during the phase I stage II of their medical program in India.

Methods: Oxacillin agar screen technique was employed to screen for MRSA. Broth macrodilution method was used to detect Minimum inhibitory concentration (MIC)values ranging between 0.25-16µg/ml. MHA broth incorporated with oxacillin antibiotic was diluted from 0.25µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml to 16µg/ml. The Cefoxitin disc diffusion test was done in order to check if BORSA isolated were *mec*A negative.

Results: Out of the 37 coagulase positive *Staphylococcus aureus* samples screened, 27 (72.97%) samples showed an MIC range between 0.25µg/ml-1.0µg/ml. Ten (27.02%) samples had the MIC range between 2µg/ml-8µg/ml; out of which 3 (8.10%) samples had an MIC value of 2µg/ml, and 3 (8.10%) samples showed MIC as 4µg/ml, while 4 (10.81%) showed the MIC to be 8µg/ml in broth dilutions. None (0%) showed MIC for 16µg/ml, and the Cefoxitin disk diffusion tests conducted showed that the 37 isolates of CoPS had zones of inhibition >21mm in diameter.

Conclusions: This study revealed the asymptomatic nasal carriage of BORSA (6.49%) and coagulase positive methicillin susceptible (17.53%) *Staphylococcus aureus*(MSSA) in healthy student volunteers. Ten (27.02%) samples that had MIC values between 2-8µg/ml showed cefoxitin susceptibility, proving the absence of the *mec*A gene; they were classified as BORSA; while 27 (72.97%) samples that were cefoxitin susceptible *mec*A negative strains showed MIC values that ranged between 0.25 µg/ml to 1.0 µg/ml, and were classified as MSSA.

Key Words: Heteroresistance, CoPS, BORSA, ORSA, MIC, MSSA, Broth macrodilution, Nasal colonization

Key Messages: Antimicrobial resistance, pre-clinical study, survey, asymptomatic nasal carriage

Corresponding Author
Santhosh D V. Dept.of Microbiology
Melaka Manipal Medical College(Manipalcampus)
International Centre for Health Sciences
Manipal - 576 104,Karnataka, India
Ph.Off:91-0820 2922634,M Fax:91-820-2571905
M-91-9886901222
Email address: daphnevincent@yahoo.com

Introduction

Therapeutic challenges posed by clinical infections caused by community-acquired and nosocomial strains of oxacillin resistant Staphylococcus aureus (ORSA) continue to be a major threat world-wide[1],[2],[3],[4],[5]. Recent outbreaks of

infectious syndromes involving oxacillin resistant strains (ORSA) and *mec A* negative borderline oxacillin resistant strains of *Staphylococcus aureus* (BORSA) have been isolated, with their MIC levels ranging from 1-4µg/ml. Possible risk factors for patients harbouring BORSA in a case control study by Balslev et. al showed that, in comparison to the controls, BORSA infected patients were more prone to severe skin infections, were more often hospitalized, and had more bed-days[6],[7]. A vast majority of ORSA and BORSA have been shown to produce β-lactamases that hydrolyse many penicillins and narrow-spectrum cephalosporins.[6],[8],[9],[10].

Initially, BORSA isolates described were non-heteroresistant strains of *Staphylococcus aureus* with oxacillin MIC ≤ 2mg/L, and subsequent isolates showed a higher oxacillin MIC ranging between 2-8mg/L.[11],[12]. The mechanism of resistance exhibited by BORSA include excessive penicillinase production, plasmid mediated inducible methicillinase, or point mutations of penicillin-binding proteins[12].The clinical implications of BORSA are still unknown. Treatment efficacy is still at a questionable stage, with a higher oxacillin MIC of 2-8mg/L. Treatment with penicillinase resistant penicillins (PRP) for BORSA with MIC ≤ 2mg/L is supposedly efficient, while high dose β-lactam/β-lactamase inhibitor combinations are found to be as effective as PRP in animal models[11],[13],[14].

Our initial study was done to determine nasal carriers of methicillin resistant Staphylococcus aureus (MRSA) from the anterior nares of pre-clinical medical students hailing from Malaysia; to see if there was any nasal carriage in the student community before they are exposed to the clinical settings in their academic postings. The nasal samples yielded no MRSA. The study further led us to determine low-high oxacillin resistant Satphylococcus aureus showing heteroresistant patterns.

Materials and Methods

Study Design and sampling

This study was approved by the institutional Kasturba hospital ethical clearance committee (KHEC) of Kasturba Medical College, Manipal, India, for the collection of samples from the student community, which included 65 males and 92 females. This study was a cohort study, designed to analyze the carrier rate of borderline oxacillin resistant *Staphylococcus aureus* in 157 pre-clinical students from Malaysia studying at Melaka Manipal Medical College, Manipal University, Manipal, India, and whose ages were between 18-22 years. Out of a total of approximately 350 students, 157 of them volunteered to participate in this study, and samples were obtained after written consent was obtained from the population under study. From the 37 Coagulase positive *Staphylococcus aureus* (CoPS) samples, further analyses were conducted to study the heterotypic resistance to oxacillin.

Specimen Collection

For the isolations, samples were collected from the anterior nares of student volunteers using sterile cotton swabs soaked in sterile saline, and samples were directly inoculated onto sheep blood agar (FI-Chemechtron, Pvt. Ltd., Bangalore, India).

Processing of specimens

Inoculated sheep blood agar plates were kept for 24 hours of incubation at 37^{o}C. Golden yellow to white, opaque, rounded, convex colonies were isolated for further biochemical analysis. Strains that were catalase positive and that which fermented mannitol were identified as Staphylococcus species.

Slide coagulase and tube coagulase test:[7] These tests were done in order to isolate coagulase positive *Staphylococcus aureus*.

DNase test: [22] DNase activity was tested by the use of DNase test agar w/ Toluidine Blue (M1041, HIMEDIA Laboratories Pvt. Ltd., Mumbai, India). The medium was prepared according to the manufacturer's instructions. Pinkish clearing around the

colonies on the DNase test medium confirmed DNase activity.

Oxacillin agar screen with 4%NaCl at a concentration of 6µg/ml was done to screen for MRSA.

Phenotypic detection of BORSA

Broth macrodilution:

Decreasing concentrations of cloxacillin (500mg; Biochem Pharmaceutical Industries Pvt. Ltd., Mumbai, India) in serial two-fold dilutions were done, ranging from 0.25-16µg/ml.[15] The antibiotic was dissolved and prepared according to the manufacturer's instructions, and was diluted in Mueller- Hinton broth, MHB (M391, HIMEDIA Laboratories Pvt. Ltd., Mumbai, India)[16] with 2% NaCl (Universal Laboratories Pvt. Ltd., Mumbai, India) in accordance with NCCLS standards (formerly called as National Committee for Clinical Laboratory Standards, now CLSI Clinical Laboratory Standards Institute). The coagulase positive Staphylococcus aureus samples that were isolated, were subcultured onto sheep blood agar (FI-Chemechtron, Pvt. Ltd., Bangalore, India) and incubated overnight for 24 hours at 37°C. CoPS were standardized to 0.5×McFarland. The positive control was a tube with MHB, without antibiotic, while the negative control was an uninoculated tube. The tubes were incubated for 48 hours at 37°C. The lowest concentration of cloxacillin that inhibited bacterial growth visualized by the lack of visual turbidity was designated as the minimum inhibitory concentration (MIC). Control strains used were *Staphylococcus aureus* MSSA strain ATCC 29213, ATCC 33592 and ATCC 43300.

Cefoxitin Disk Diffusion Method

[18]Mueller-Hinton agar (MHA) plates were inoculated with 0.5×McFarland standard suspension of the 37 samples that showed heterotypic oxacillin resistance with MIC ranging from 2-16µg/ml, by streaking over the agar surface. cefoxitin disks of 30µg (CT01198, Oxoid Ltd., Hampshire, England) were placed onto the surface of the MHA and incubated for 24 hours at 37°C. cefoxitin susceptible or *mec*A negative *Staphylococcus aureus* strain ATCC 29213 was used as a quality control strain. Cefoxitin inhibition zone diameter >21mm was recorded for all the test strains.[19]

Statistical analysis: Chi-square test was applied to check whether the distribution was the same between MRSA, BORSA and MSSA.

Results

Out of the 37 coagulase positive Staphylococcus aureus samples screened, 27 (72.97%) samples showed an MIC range between 0.25µg/ml-1.0µg/ml, 10 (27.02%) samples had an MIC range between 2µg/ml-8µg/ml; out of which 3 (8.10%) samples had an MIC value of 2µg/ml, 3 (8.10%) samples showed MIC as 4µg/ml, while 4 (10.81%) showed an MIC of 8µg/ml in broth dilutions. None (0%) showed MIC for 16µg/ml, and the cefoxitin disk diffusion tests conducted, showed that the 37 isolates of CoPS had zones of inhibition >21mm in diameter
[Table /Fig 1].

[Table/Fig 1]: Out of a total of 37 isolates of CoPS, 10 were conclusively BORSA with cefoxitin disc susceptibility being greater than 21mm in diameter, indicating that the samples were mecA negative. Significantly higher proportion of MSSA was observed

Asymptomatic nasal carriage of BORSA, MSSA among the student population from Malaysia

No. of samples (CoPS)	MIC (µg/ml)	Nasal carriage (%)	Cefoxitin disc diffusion	*mec*A
MSSA				
14	0.25	37.83	>21mm	-
8	0.5	21.62	>21mm	-
5	1.0	13.51	>21mm	-
BORSA				
3	2	8.10	>21mm	-
3	4	8.10	>21mm	-
4	8	10.81	>21mm	-
MRSA				
0	16	0	-	-

(chi-square value (1df)=30.2; P<0.001)

All samples of CoPS were susceptible to oxacillin on agar screen at a concentration of 6µg/ml, while in broth macrodilution, heterotypic resistance patterns to oxacillin with MICs ranging between 0.25% to 1.0% and MICs between 2-8µg/ml, were isolated.[17] Ten of the coagulase positive Staphylococci that showed an heterotypic resistance to oxacillin with MICs between 2-8µg/ml, were classified as borderline oxacillin resistant *Staphylococcus aureus* (BORSA), and the cefoxitin disc diffusion test conducted for these 10 samples confirmed the absence of the *mec*A gene, as their zones of inhibition were all found to be

greater than 21mm in diameter, and characteristically these 10 samples also did not show growth on the oxacillin agar screen test.

Discussion

This study goes to show that from a group of 157 pre-clinical Malaysian students screened, 10 of them were healthy nasal carriers of BORSA. A retrospective study of their clinical histories did not reveal any hospitalizations or severe infections. They did not show any symptoms of BORSA infections, although the clinical implications of BORSA in non-symptomatic carriers, is still debated. The rate of nasal colonization of BORSA in a young Malaysian student population sampled, revealed to be as low as 6.36%, while the percentage of nasal carriage of coagulase positive methicillin susceptible strains was 17.19%. None had any history of dermatological problems; showing that they were asymptomatic.

The phenotypic tests that were done for the detection of MRSA and the subsequent evaluation through the cefoxitin disk diffusion results were in accordance with the standard protocol carried out, proving that there were no nasal carriers for *mec*A positive MRSA carriers, and that the phenotypic detection of BORSA through broth macrodilution for MICs between 2-8µg/ml was also found to be in accordance with the cefoxitin disk diffusion test that showed zones of inhibition with diameters measuring more than 21mm. Studies have proven the efficacy of cefoxitin to be far superior than oxacillin in sensitivity and specificity[20]. BORSA strains are not well detected by the oxacillin agar screen technique. In our study for the isolation and detection of MRSA in oxacillin agar screen technique; BORSA strains did not show up, but were detected by the broth macrodilution technique.

BORSA strains lack the presence of PBP2a, and they are said to possess normal PBPs in contrast to those of the ORSA strains.[21] The low level of oxacillin resistance in these strains is thought to be due to their hyperproduction of extracellular β-lactamase [22], but according to Tomasz et al.[23], the PBPs of certain BORSA strains may show moderate affinities to methicillin, and such strains have been termed as MODSA (modified PBPs) in order to distinguish them from the normal PBP containing BORSA strains.

Although BORSA does not show even a single differentiating trait to distinguish it from the MSSA or MRSA;[24] they have been shown to be related due to the presence of 94/96 phage types, a high degree of genetic relatedness, and a 17.2kb plasmid that renders its capacity to hyperproduce β-lactamase. This feature is said to be the only characteristic trait that denotes the BORSA phenotype[25],[26] apart from the production of a methicillinase. Some authors state that the amino acid substitutions to a point mutation in PBP2 is the factor contributing to the BORSA phenotype[27].

Several BORSA isolated, were from dermatological conditions from previous studies; but our study revealed that the presence of heterotypic oxacillin resistant strains of BORSA could be found as nasal colonizers without any predisposing symptoms or clinical conditions. The tests employed as agar screen for oxacillin resistance did not allow the growth of the 10 BORSA strains, and these could only be detected through MIC values correlating with cefoxitin disc susceptibilities, indicating that this could be used in the routine laboratory detection for these strains and helps to avoid reporting of false oxacillin susceptibility patterns due to the heterogeneous resistance shown by Staphylococci. These phenotypic methods could be employed in laboratories that lack facilities for genotypic detection techniques.

Healthy reservoirs in the community are a potential source of infection. It should be made mandatory to screen for nasal carriage of heterotypic resistant strains of Staphylococcus aureus, especially in pre-clinical medical students hailing from other countries that could become healthy carriers

in the community, and could pose a risk in transmitting the resistant strains to the hospitals where they will be exposed during their clinical phase of their medical degree. This could help check the spread of community-acquired and hospital acquired infections through reservoirs in the community. Screening and survey of students prior to their entry into the hospital environment will help in restriction of the rapid spread of heteroresistant strains of Staphylococci, and therefore proper protocols for the eradication of nasal carriage of BORSA should be instituted.

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References

[1] Lance H and Arnold SB. ß-lactam-ß-lactamase inhibitor combinations are active in experimental endocarditis caused by ß-lactamase producing Oxacillin-resistant Staphylococci Antimicrob. Agents Chemother. 1991;35:685-90.

[2] Cremieux AC., Maziere B., Vallois JM, Ottaviani M., Azancott, A, Raffoul, A., Bouvet J, Pocidalo J and Carbon C. An outbreak of infections caused by strains of Staphylococcus aureus resistant to methicillin and aminoglycocides. I. Clinical studies. J.Infect.Dis. 1979;139: 273-79.

[3] Hackbarth CJ and Chambers HF. Methicillin-resistant staphylococci: detection methods and treatment of infections. Antimicrob. Agents Chemother. 1989;33:995-99.

[4] Sorrell TC, Packham D.R., Shanker S., Foldes, M., and Munro, R. Vancomycin therapy for methicillin-resistant Staphylococcus aureus. Ann. Intern. Med. 1982; 97: 344-50.

[5] Tomasz A., Drugeon HB, De Lancaster HM, Jabes D, McDougall L, and Bille J. New mechanism for methicillin resistance in Staphyococcus aureus: clinical isolates that lack the PBP2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. Antimicrob. Agents Chemother. 1989;33:1869-74.

[6] Massanari RM, Pfaller MA, Wakesfield DS, Hammons GT, McNut LA, Woolson RF, and Helms CM. Implications of acquired oxacillin resistance in the management and control of Staphylococcus aureus infections. J. Infect. Dis. 1988; 158: 702-9.

[7] Balslev U, Bremmelgard A, Svejgaard E, Havstreym J, Westh H. An outbreak of oxacillin-resistant Staphylococcus aureus (BORSA) in a dermatological unit. Microbe. Drug. Resist.2005; 11: 78-81.

[8] McDougal LK, and Thornsberry C. The role of ß-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. J Clin. Microbiol. 1986;23:832-39.

[9] Montanari MP, Tonin E, Biavasco F, and Varaldo PE. Further characterisation of borderline methicillin-resistant Staphylococcus aureus and analysis of penicillin-binding proteins. Antimicrob Agents. Chemother. 1990;34:911-13.

[10] Sierra-Madero JG, Knapp C, Karaffa C, and Washinton JA. Role of ß-lacatamase and different testing conditions in oxacillin-borderline-susceptible staphylococci. Antimicrob. Agents. Chemother. 1988;32:1754-57.

[11] Lee N, Clive SC, Grace L, Rebecca L, Edman L, Raymond L, Margaret I. Community case of methicillin-resistant Stpahylococcus aureus. Letters. Emerg Infect. Dis. 2006;12:172-74.

[12] Chambers HF, Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Molec. Biolog. Rev. 1997;10:781-91.

[13] Varaldo PE. The "borderline methicillin-susceptible" Staphylococcus aureus. J. Antimicrob. Chemother. 1993;31:1-4.

[14] Hirano L, Bayer AS. Beta-lactam-beta-lactamase inhibitor combinations are active in experimental endocarditis caused by beta-lactamase-producing oxacillin-resistant staphylococci. Antimicrob. Agents. Chemother. 1991;35:685-90.

[15] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th edn. Approved Standard M7-A5. Wayne, PA:NCCLS, 2000.

[16] National Committee for Clinical Laboratory Standards. Protocols for evaluating dehydrated Muller-Hinton agar. Approved Standard M6-A. Wayne, PA: NCCLS, 1996.

[17] Louie L, Matsumura SO, Choi E Louie M, Simor A.E. Evaluation of three rapid tests for the detection of methicillin resistant Staphylococcus aureus. J.Clin.Microb. 2000;38: 2170-73.

[18] Boutiba-Ben Boubaker I, Ben Abbes R, Ben Abdullah H, Mamlouk K, Mahjoubi F, Khammoun A. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant Staphylococcus aureus. Clin. Microb. Infection. 2004;10:762-65.
[19] Mougeot C, Guillaumat-Tailliet J, Libert JM. Staphylococcus aureus:new detection of intrinsic resistance using the diffusion method. Path Biol 2001; 49: 199–204.
[20] Smyth RW. and Kahlmeter G. Mannitol salt agar-cefoxitin combination as a screening medium for methicillin-resistant Staphylococcus aureus. J.Clin.Microb. 2005;43: 3797-99.
[21] Chambers HF, Archer G and Matsuhashi M. Low –level methicillin resistance in strains of Staphylococcus aureus. Antimicrob. Agents Chemother. 1989;33:424-428.
[22] McDougal LK, and Thornsberry C. The role of ß-lactamase in stpaylococcal resistance to penicillin-resistant penicillins and cephalosporins. J.Clin.Microbiol. 1986;23: 832-39.
[23] Tomasz A, Drugeon HB, De Lancastre HM, Jabes D, McDougall L, and Bille J. New mechanism for methicillin resistance in Staphylococcus aureus: clinical isolates that lack the PBP2a gene and contain normal Penicillin-binding proteins with modified penicillin-binding capacity. Antimicrob. Agents Chemother. 1989;33:1869-74.
[24] Barg N, Chambers H & Kernodle D. Borderline susceptibility to antistaphylococcal penicillins is not conferred exclusively by the hyperproduction of beta-lactamase. Antimicrob Agents Chemother 1991;35, 1975–79.
[25] Massidda O, Montanari M P, Mingoia M & Varaldo PE. Borderline methicillin-susceptible Staphylococcus aureus strains have more in common than reduced susceptibility to penicillinase-resistant penicillins. Antimicrob Agents Chemother (1996). 1996;40:2769–74.
[26] McMurray L W, Kernodle D S& Barg N L. Characterization of a widespread strain of methicillin-susceptible Staphylococcus aureus associated with nosocomial infections. J Infect Dis. 1990;162: 759–62.
[27] Jeya N, Mark J Lee S, Lisa L, Latha J, Andrew ES, Marie L and Martin J M. Identification of different clonal complexes and diverse amino acid substitutions in penicillin-binding protein 2 (PBP2) associated with borderline oxacillin resistance in Canadian Staphylococcus aureus isolates. JMed Microbiol 2006;55: 1675-83