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Original Research Paper

Anatomy

OXACILLIN SCREENING FOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Introduction: Methicillin resistant staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that is resistant to a large group of antibiotics. Study was aim to screen the isolated S. aureus for Oxacillin resistance by Oxacillin screen agar.

Methods: Clinical specimens obtained from patients in various departments of BPKIHS, and submitted to department of Microbiology for culture and sensitivity were included. Among 90 isolated SA was screened by using cefoxitin disc as per Kirby-Bauer's disc diffusion method. MRSA were again screened with the Oxacillin screening agar.

Results: Out of these 90; 23(25.56 %) were found to be MRSA and rest 67(74.44%) were Methicillin sensitive staphylococcus aureus (MSSA). Then the Oxacillin screening test showed that 32 isolates were sensitive to oxacillin (35.55%) while 58 were resistant to oxacillin (64.45%).

Conclusion: In this study 26% MRSA was found, which indicated the alarming threats to use the beta lactam antibiotics.

KEYWORDS : MRSA, Oxacillin, Cefoxitin

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a circular, aerobic or facultative anaerobic, Gram positive bacterium. *S. aureus* is one of the most common bacterial agents which cause a range of illness from minor skin infections to life threatening diseases such as sepsis food poisoning. Over recent years *S.aureus* has emerged as one of the significant pathogens associated with both the community acquired and nosocomial infection usually septicaemia, wound sepsis, food poisoning pneumonia, septic arthritis, osteomyelitis, and toxic shock syndrome[1]. On blood agar they form characteristic golden (Latin *aureum*) or white colonies. They produce catalase; coagulase and an extracellular cell clumping factor, and some strains produce capsule[2]. MRSA is defined as a strain of *S. aureus* that is resistant to a large group of antibiotics called beta lactams which includes penicillin and cephalosporin. The resistant in MRSA is due to the expression of penicillin binding protein (PBP2a) encoded by *mecA* genes[3], which is located on the Staphylococcus cassette chromosome (SCC).

Since the first report of methicillin resistant *Staphylococcus aureus* as a major nosocomial pathogen in 1960s, the incidence of infections caused by this organism continues to increase worldwide[4]. The cost effective but fast high sensitivity screening method for MRSA in hospital setting is a must. However, there have been expensive PCR based methods for direct detection of MRSA, which may be cost inhibitive[5-8].

The Oxacillin Resistant Screening Agar (ORSA) was recently used for the screening of specimens for MRSA in a hospital setting [9]. Therefore, this work was carried out to evaluate (i) the sensitivity of the procedure for the recovery of MRSA from patient specimens by using ORSA base medium alone as a primary culture medium or as an enrichment broth medium for subculture, (ii) the proportion of samples that do not contain MRSA but that show the growth of blue colonies on ORSA medium and (iii) the optimal incubation time. Present study was aim to isolate and identify *S. aureus* from clinical specimens and to screen the isolates for Oxacillin resistance by Oxacillin screen agar. Increasing spread of poly resistant strains of *Staphylococcus aureus* is a problem of global extent. The treatment of MRSA is to be done with fifth generation cephalosporin which is costly and still not marketed in third world country like ours. MRSA is also implicated in health care associated infections and it is in the increasing scenario that it is transferred from the clinician to other individuals. Thus the study finds it an utmost necessity to detect profile of MRSA in the clinical isolates.

MATERIALS AND METHODS:

In this cross sectional study specimens were obtained during April 2015 to July 2015 from various patients of clinical departments of B.P. Koirala Institute of Health Sciences, and submitted to department of Microbiology for culture and sensitivity were included. Out of the 90 *S. aureus*, most samples were collected from OPD department (33), while second most samples from pediatric emergency department (16) followed by other departments.

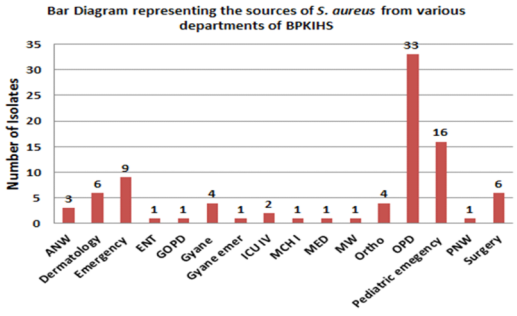
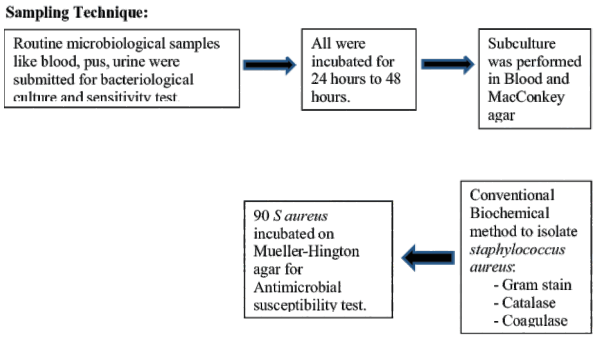


Figure 1: Bar diagram showing the origin of isolates from various departments

Among 90 isolated SA was screened by using cefoxitin (Cx) disc as per Kirby-Bauer's disc diffusion method. Then the MRSA were again screened with the Oxacillin screening agar.



Antibiotics used in antimicrobial susceptibility test for S. aureus

- Ampicillin 10 µg
- Amikacin 30 µg
- Cefoxitin 30 µg
- Ceftriaxone 30 µg
- Ciprofloxacin 5 µg
- Azithromycin 15 µg
- Cotrimoxazole 23.75 µg
- Linezolid 30 µg
- Vancomycin 30 µg

Interpretive criteria (in mm) for cefoxitin disk diffusion test:

	Sensitive	Resistant
Staphylococcus aureus	≥ 22 mm	≤ 21 mm

Oxacillin Screening Method:

All *S. aureus* were inoculated in oxacillin screen agar plates with 4% NaCl and 6µg/ml of oxacillin. → Incubated for 24 hours at 35^0 Celsius. → Plates were observed carefully.

In data analysis frequency mean, median and standard deviation was calculated. Statistical analysis of the risk factor association with SA and MRSA colonization was calculated using *Chi square* test.

RESULTS

The gender segregation showed that 51.11% patients were male (46) and rest were 48.89% were female (44). The prevalence of MRSA among female which was found to be statistically significant at P value of 0.021 suggesting that females were more likely to be infected with MRSA. The MRSA were again screened with the Oxacillin screening agar and this test showed that 32 isolates were sensitive to oxacillin (35.55%) while 58 were resistant to oxacillin (64.45%). The mean age of the patients (26.03) ranged from 1 day to 83 years, with SD ± 22.72 years. Out of total isolates samples, 36 (40%) were from Inpatients departments while 54 (60%) were from outpatient department.

MRSA by Cefoxitin

Again among the ninety isolates, which were identified as *S. aureus*, were subjected to cefoxitin sensitivity test. The zone of inhibition of cefoxitive is ≥22 mm. Among the 90 samples 23 (25.56%) samples were methicillin sensitive (MRSA) and 67 (74.44%) were methicillin resistant (MSSA).

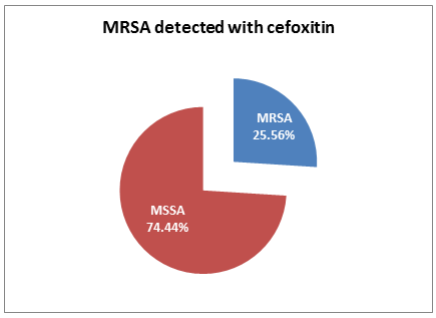


Figure 2: Pie chart showing MRSA detection with cefoxitin.

Oxacillin screening test done on MRSA

Out of the isolated 90 samples, 32 (35.56%) isolates were oxacillin sensitive while 58 (64.44%) isolates were oxacillin resistant. The following pie chart shows the oxacillin sensitivity and resistivity.

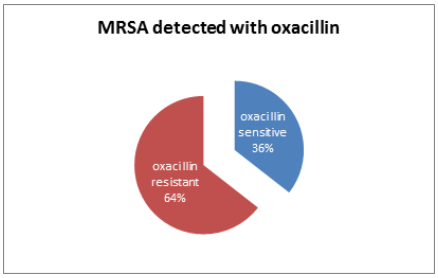


Figure 3: Pie chart showing MRSA detection with oxacillin.

DISCUSSION

Community-associated methicillin-resistant Staphylococcus aureus (MRSA) is emerging as a leading cause of skin and soft-tissue infections in many parts of the world. Recently, many cases of MRSA infection have been reported in healthy community individuals with no traditional risk factor. MRSA carriage prevalence was found to be 26% (23/90) in the present study. Other community-based studies have also found similar MRSA prevalence. The overall MRSA carriage rate of 8.5% was observed from South India. Munckhof et al. found a prevalence of only 0.7% among 699 patients in Queensland, Australia. A higher propensity was observed amongst MRSA strains reported in USA (24.15%). Taiwan and Zaria, Nigeria, reported a prevalence of 13.6% and 14.85%, respectively, from anterior nares of healthy population, adults and school pupils. In our study 16 of the 23 MRSA isolates were from females, although it was not statistically significant. A study from South India reported males to be the major carriers (15/118, 12.4%). Statistical analysis of the risk factor association with SA and MRSA colonization was calculated using the Chi-square test. The present study shows ciprofloxacin resistance towards SA to be 21.11% which is in similar to the results observed by Chatterjee et al. with 2.4%. Antibiotic sensitivity pattern of the MRSA isolates shows 8% resistance to ciprofloxacin and 100% sensitivity to vancomycin. These findings are in sharp contrast with a study from Delhi where 60% MRSA were resistant to ciprofloxacin along with 100% sensitivity towards Vancomycin, but similar to the results by Chatterjee et al with resistance to ciprofloxacin being 12.5%. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading pathogen of healthcare-associated infections in intensive care units (ICUs). Routine active screening for MRSA and decolonization in hospital settings is associated with a decrease in MRSA infections, mortality and medical cost in settings with a high MRSA infection rate. Both MRSA carriers and non-carriers can benefit from a routine active MRSA screening and decolonization program. In settings where MRSA is endemic, MRSA carriage rate drops after implementation of the active screening and decolonization program. The limitations of the study were; the small samples size and no molecular techniques were used for the true confirmation of isolates.

CONCLUSION

This study documented 26% of MRSA circulation in our set up which indicated the alarming threats to use the beta lactam antibiotics. However, this number should be verified by other recent techniques which could provide the real estimation of the MRSA. In addition, regular surveillance of MRSA in routine set up and antibiotic treatment guidelines should be implemented to prevent such type of potential threat of antibiotic resistance.

REFERENCES

1. Bannerman TL. Staphylococci, Micrococcus and other catalase-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JM et al. eds. Manual of Clinical Microbiology, 8th edn. Washington: American Society for Microbiology Press,2003; 384–404
2. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with β-lactam resistance in Staphylococcus aureus Journal of Bacteriology 1984; 158: 513–6
3. Reynolds PE, Brown DF. Penicillin-binding proteins of β-lactam-resistant strains of Staphylococcus aureus. Effect of growth conditions. FEBS Letters 1985; 192:28–32
4. Safdar, N., L. Narans, B. Gordon, and D. G. Maki (2003): Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant Staphylococcus aureus: a prospective study comparing 32 methods. J. Clin. Microbiol. 41:3163-3166.
5. Blanc D. S., Pittet D., Ruef C., Widmer A., Muhlemann K., Petignat C., Harbarth S., Auckenthaler R., Bille J., Frei R., Zbinden R., Peduzzi R., Gaia V., Khamis H., Bernasconi E., and Francioli. P. (2002): Epidemiology of methicillinresistant Staphylococcus aureus: results of a nationwide survey in Switzerland. Swiss Med. Wkly. 132:223-229. 2
6. Francois, P., Pittet D., and Bento M., Pepey B., Vaudaux P., Lew D., and Schrenzel J. (2003): Rapid detection of methicillin resistant Staphylococcus aureus directly from sterile or nonsteri le clinical samples by a new molecular assay. J. Clin. Microbiol. 41:254-260. 3.
7. Huletsky A., Gagnon F., Ross Bach V., Truchon K., Picard F. J., and Bergeron M. G. (2002): Less than one-hour detection of methicillin-resistant Staphylococcus aureus directly from nasal swabs by real-time PCR using the smart cycler. Clin. Microbiol. Infect. 8(Suppl. 1):85. 4
8. Louie L, Goodfellow J., Mathieu P., Glatt A., Louie M. and Simor A. E.(2002): Rapid Detection of Methicillin-Resistant Staphylococci from Blood culture Bottles by Using a Multiplex PCR Assay. J. Clin. Microbiol, Vol. 40, No. 8p. 2786–2790.
9. Blanc D. S., Wenger A., and Bille J. (2003): Evaluation of a Novel Medium for Screening Specimens from Hospitalized Patients to Detect Methicillin-Resistant Staphylococcus aureus. J Clinil Microbiol, August 2003, p. 3499-3502, Vol. 41, No. 8.