

Original Article

DRUG RESISTANCE PATTERNS OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN TERTIARY CARE CENTER OF SOUTH INDIA

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ABSTRACT

Objectives: *Staphylococcus aureus* were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. The current study was undertaken to provide data for empirical selection of appropriate antibiotics for the treatment of diseases caused by *S. aureus*.

Methods: Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients were selected for study from June 2012 to June 2013. *Staphylococcus aureus* were identified by various biochemical tests and antimicrobial susceptibility testing of the isolates were performed by Kirby Bauer disc diffusion method. Detection of the MRSA was done by Oxacillin disc diffusion method.

Results: A total of 137 isolates of *S. aureus* were obtained over duration of 12 months. These included isolates from the sample of pus, urine, sputum, body fluids. Out of 137 *S. aureus* strains isolated, 62 (45.3%) were identified as MRSA and 75 (54.7%) were identified as MSSA based on oxacillin disk diffusion method. Anti-biograms revealed the high level of resistance among MRSA isolates when compared to MSSA isolates. The most effective agent against MRSA isolates was linezolid (96.8% sensitive), followed by tetracycline (90.9%) and piperacillin/tazobactam (80.6%).

Conclusion: The prevalence of MRSA in our hospital was high. Therefore to reduce the incidence of infections due to MRSA, we suggest implementation of the strict antibiotic policy guidelines and continuous monitoring of antibiotic susceptibility patterns of such pathogens.

Keywords: Antibiotic susceptibility pattern, Drug resistance, Methicillin resistant *Staphylococcus aureus* (MRSA), antibiotic resistance, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus was first described by Sir Alexander Ogston in 1882. This centuries-old pathogen still causes significant morbidity and mortality despite huge advances in medical care. Indeed, infections due to *S. aureus* continue to grow in number and complexity as a consequence, ironically, of advances in patient care and of its ability to adapt to a changing environment [1, 2].

Methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged after the introduction of methicillin into the clinical practice [3]. Methicillin resistant *Staphylococcus aureus* (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. Infected and colonized patients provide the primary reservoir and transmission is mainly through hospital staff [4].

The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalisation in an intensive care unit. With the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many strains of MRSA exhibit resistance to beta-lactams and aminoglycosides [4]. The worst feature of MRSA has been simultaneous drug resistance to many of the antibiotics, chronic carrier stage among health care workers and greater resistance of the strains [5]. The current study was undertaken to provide data for empirical selection of appropriate antibiotics for the treatment of diseases caused by *S. aureus*.

MATERIALS AND METHODS

Source of data

The study was conducted in the Department of Microbiology, Shri B. M Patil Medical College Hospital, Bijapur. *S. aureus* isolated from all the clinical samples that came to the microbiology department formed the material for study.

Ethical clearance and consent

The study was conducted after obtaining the ethical clearance from institutional ethical committee.

Method of collection of data: (including sampling procedure)

Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients attending Shri B M Patil Medical College and Hospital were selected for study for a period of one year from June 2012 to June 2013.

Statistical analysis

Data was analyzed by

- 1) Diagrammatic representation
- 2) Proper statistical tests like chi square test etc.

Inclusion criterion

Samples which yielded *S. aureus* were included in the study.

Exclusion criterion

Samples which did not yield *S. aureus* were excluded from the study.

Specimens were screened by preliminary Gram's stain and then inoculated on 10% sheep blood agar and MacConkey's agar. *S. aureus* was identified by conventional techniques [6]. Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method using following discs. penicillin-G (10 unit); cloxacillin (30µg); cephalixin (30µg); cefuroxime(30 µg); tetracycline (30µg);erythromycin (15µg); gentamycin (10µg); ciprofloxacin (5µg); pefloxacin (5µg); Cefoperazone/salbactam(75 µg/30 µg) piperacillin/tazobactam(100µg/10 µg); amoxicillin/clavulanic acid (20 µg/10 µg); azithromycin(15µg); linezolid (15µg). Finally, the data were recorded and analyzed at the completion of

the study as per recommendations of the CLSI [7]. *S. aureus* ATCC 29213 were used as the reference strain for the standardization of antibiotic susceptibility testing. Detection of the MRSA were done by Oxacillin disc diffusion method [8,9] All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using oxacillin discs (1µg). The isolates will be considered methicillin resistant if the zone of inhibition was 10 mm or less.

RESULTS

A total of 137 isolates of *S. aureus* were obtained over duration of 12 months. These included isolates from the sample of pus, urine, sputum, body fluids (table 1).

Table 1: Distribution of *S. aureus* isolates in various clinical specimens

Sample	Total <i>S. aureus</i>	MRSA	MSSA
Pus	103	49	54
Urine	15	6	9
Ear swab	9	3	6
Sputum	6	1	5
Others	4	3	1
Total	137	62	75

Out of 137 *S. aureus* strains isolated, 62 (45.3%) were identified as MRSA and 75 (54.7%) were identified as MSSA based on oxacillin disk diffusion method. Eighty five (62.1%) were isolated from male patients and 52 (37.9%) from female patients. Out of total 137 patients, infection was nosocomially-acquired in 103 (75.2%) patients and 34 (25.8%) were community-acquired. Of all samples with *S. aureus*, majority 67 (48.9%) were obtained from surgery department, followed by 21 (15.3%) from ENT department (table 2)

Table 2: Distribution of *S. aureus* isolates in various clinical departments

Department	No. of <i>S. aureus</i> isolates	Percentage
Surgery	67	48.9
ENT	21	15.3
Medicine	12	8.76
OBG	11	8.03
Orthopedic	10	7.3
SVD	9	6.57
Others	7	5.11
Total	137	100

Anti-biograms of *S. aureus* (MRSA and MSSA) isolates to 14 antimicrobial agents including linezolid, cephalosporins, aminoglycosides, and fluoroquinolones are presented in (table 3). The table revealed high level of resistance among MRSA isolates when compared to MSSA isolates. The most effective agent against MRSA isolates was linezolid (96.8% sensitive), followed by tetracycline (90.9%) and piperacillin/tazobactam (80.6%).

Table 3: Antibiotic susceptibility pattern of *S. aureus* isolates

Antibiotics	Sensitivity in	
	MRSA	MSSA
Penicillin-G	00	9.33
Cloxacillin	58.1	36.7
Cephalexin	46.8	94.7
Cefuroxime	59.7	88
Tetracycline	90.9	90.7
Erythromycin	51.6	60
Gentamycin	75.5	78.7
Ciprofloxacin	25.8	21.3
Pefloxacin	37.1	25.3
Azithromycin	53.2	65.3
Cefoperazone/salbactam	72.6	98.77.4
Pepericillin/tazobactam	80.6	94.7
Amoxicillin/clavulanic	27.4	46.7
Linezolid	96.8	100

DISCUSSION

With the discovery of penicillin in 1940 the incidence of bacterial infection decreased worldwide until *S. aureus* began producing an enzyme, beta-lactamase, that destroys penicillin. Increasing resistance to penicillin has led to the development of semi-synthetic groups of penicillin such as methicillin, that are resistant to many genetic variations of the beta-lactamase enzyme. For years, infection by *S. aureus* was controlled using methicillin and its analogues. However, in 1961 the first strain of MRSA was isolated. Since then, MRSA has been found worldwide [10, 11].

In this study, the prevalence of MRSA was more among elderly people, it was not statistically significant ($P>0.05$). This is because the elderly people constitute the large proportion of our hospital populations and reduced immunity in these people to fight against infection in general. Similar results were seen in the study conducted by Madani [12] and Buzaid *et al.* [11] who reported that MRSA affected all age groups.

Some investigators have reported no significant correlation with gender in MRSA infection, in Southern India, it was found more frequently in male patients [13,14]. Our study observed that the prevalence of MRSA as a whole did not vary significantly by gender but were more frequent among male patients, but it was statistically not significant ($P\text{ value}>0.05$). Similarly Buzaid *et al.* [11], and Charlebois *et al.* [15], observed no differences and the prevalence rates among *S. aureus* from females and males. These authors have reported that gender was not identified to be a risk factor for the acquisition or colonization of MRSA.

In our study, 45 % of the total isolates tested were methicillin-resistant, as defined by resistance to oxacillin. This is comparable to other studies conducted across India and other parts of the world.[3,11,16-18]. Contrary to this results, low prevalence of MRSA (10-12%) were reported in Nepal [19]and Trinidad and Tobago [20] Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group's report, the prevalence of MRSA varies from 22% to 68% in Indian hospitals [21]. These studies stress the need to monitor the prevalence of MRSA continuously in a given population, because it can vary from region to region [19].

In the present study, majority of the isolates were from pus sample (75.2%) which was consistent with suppurative nature of Staphylococcal infections and from the surgery department (48%). Similar findings were reported by Simor *et al.*, [22], Akpaka *et al.*,[20]. The reasons the higher proportion of MRSA cases among surgical patients may be related to the poor environmental cleaning, operation theatre surveillance and infection control measures of hospitals in Indian setup and also because of high usage of antibiotics as noted by Swanston *et al.* [23].

The present study revealed the high level of resistance among MRSA isolates when compared to MSSA isolates. Many of the isolates were resistant to commonly used antistaphylococcal agents. The most effective agent against MRSA isolates was linezolid (96.8% sensitive), followed by tetracycline (90.9%) and piperacillin/tazobactam (80.6%), and these agents might be used to treat MRSA infections if isolates are shown to be susceptible. Among above agents that might be considered for treatment of MRSA infections, linezolid should be considered as the reserve drug and used with caution. Resistance to quinolones i.e. ciprofloxacin (74.2%) and pefloxacin (62.9%) were high in this study. This is comparable to the study done by Sanjana *et al.* [16], and Saikia *et al.*[24] in Nepal and Assam respectively. Resistance to cephalixin (54%) was also much higher in this study. This is consistent with to the study carried out by Sanjana *et al.* [16], and Kumari *et al.* [25], who reported the similar resistant rate to cephalixin.

CONCLUSION

The current study was the first to report on the prevalence of MRSA in the tertiary hospital in this part of India. This study showed that the prevalence of MRSA in our hospital was high. Therefore to reduce the incidence of infections due to MRSA, we suggest implementation of the strict antibiotic policy guidelines and continuous monitoring of antibiotic susceptibility patterns of such pathogens.

CONFLICT OF INTERESTS

Declared None

REFERENCES

- Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 2008;46(Suppl 5):S344–S9.
- Metri BC, Peerapur BV, P Jyothi. Comparison of antimicrobial resistance pattern of hospital-and community-acquired Methicillin resistant *Staphylococcus aureus*. J Chem Pharm Res 2014;6:201-5.
- Anand KB, Agrawal P, Kumar S, K Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA. Indian J Med Microbiol 2003;27:27-9.
- Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol 2003;21:49-51.
- Vermaa S, Joshii S, Chitniss V, Hemwanii N, Chitniss D. Growing problem of methicillin resistant staphylococci-Indian scenario. Indian J Med Sci 2000;54:535-40.
- Betty AF, Daniel FS, Alice SW. (Editors) *Staphylococcus, Micrococcus and similar organisms*, Chapter 19. In: Baily and Scott's Diagnostic Microbiology. 11th edn. (Mosby Inc: St. Louis); 2002. p. 284.
- Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; 16th information supplement (M100-S16). Clinical and Laboratory Standards Institute, Wayne, Pa; 2006.
- Isenberg HD. editor. Clinical microbiology procedures hand book. 2nd ed. Washington DC: ASM Press; 2004.
- Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Townner KJ, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2005;56:1000-18.
- Jones RN. Key considerations in the treatment of complicated *Staphylococcal* infections. Clin Microbiol Infect 2008;14:3-9.
- Buzaid N, Elzouki AN, Taher I, Ghenghesh KS. Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary surgical and trauma hospital in Benghazi, Libya. J Infection Developing Countries 2011;5:723-6.
- Madani TA. Epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* (MRSA) at the University Hospital, Jeddah, Saudi Arabia. J KAU. Med Sci 2008;10:3-12.
- Mathanraj S, Sujatha S, Sivasangeetha K, Parija SC. Screening for methicillin-resistant *Staphylococcus aureus* carriers among patients and health care workers of a tertiary care hospital in south India. Indian J Med Microbiol 2009;27:62-4.
- Kali1 A, Stephen S, Umadevi S, Kumar S, Joseph NM, Srirangaraj S. changing trends in resistance pattern of methicillin resistant *Staphylococcus aureus*. J Clin Diagn Res 2013;7:1979-82.
- Charlebois ED, Bangsberg DR, Moss NJ, Moore MR, Moss AR, Chambers HF, et al. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin Infect Dis 2002;34:425-33.
- Sanjana RK, Shah R, Chaudhary N, Singh YI. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) in CMS-teaching hospital: a preliminary report. J College Med Sci Nepal 2010;6:1-6.
- Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistant *S. aureus*. Indian J Pathol Microbiol 2010;53:79-82.
- Fluit AC, Wielders CLC, Verhoef JF, Schmitz J. Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the european SENTRY study. J Clin Microbiol 2001;39:3727-32.
- Subedi S, Brahmadathan KN. Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Nepal. Clin Microbiol Infect 2005;11:235-7.
- Akpaka PE, Kissoon S, Henry W, Swanston WH, Monteil M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates from Trinidad & Tobago. Ann Clin Microbiol Antimicrob 2006;5:16.
- Indian network for surveillance of antimicrobial resistance group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence and susceptibility pattern. Indian J Med Res 2013;137:363-9.
- Simor AE, Ofner-Agostini M, Bryce E, Green K, McGee A, Mulvey A, et al. The canadian nosocomial infection surveillance program health Canada: Evolution of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: 5 years of national surveillance. CMAJ 2001;165:21-6.
- Swanston WH. Methicillin-resistant *Staphylococcus aureus*. West Indian Med J 1999;48:20-2.
- Saikia L, Nath R, Choudhury B, Sarkar M. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in Assam. Indian J Crit Care Med 2009;13:156-8.
- Kumari N, Mohapatra TM, Singh YI. Prevalence of Methicillin resistant *Staphylococcus aureus* in a tertiary-care hospital in Eastern Nepal. J Nepal Med Assoc 2008;47:53-6.