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Antibiotic resistance patterns of *Staphylococcus aureus*: A multi center study from India

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ABSTRACT

Chemotherapy and emergence of drug resistance strains of *Staphylococcus aureus* is receiving serious threats, due to the origin and spread of hospital and community acquired MDR strains. The present study reports the prevalence of antibiotic resistance among *Staphylococcus aureus* isolated from clinical samples from different cities of India. Antibiotic sensitivity was performed by Kirby-Bauer disk diffusion method and minimum inhibitory concentrations were determined for vancomycin and methicillin according to CLSI (2014) guidelines. A total of 212 *S. aureus* were obtained from different samples such as pus, blood, urine. The antibiogram of these isolates indicated widespread resistance to various groups of antibiotics ranging from a minimum of 10.13% against Phenicols (Chloramphenicol) to a maximum of 97% against Penicillin and 44.8% isolates were MRSA and alarmingly 10.84% were VRSA. Most of the MRSA isolates showed inducible Clindamycin resistance. Widespread prevalence of MDR patterns, increasing incidence of MRSA and VRSA calls for exploration of alternative medicines and new approaches to combat *Staphylococcal* infections.

1. Introduction

Staphylococcus aureus is one of the rapidly emerging and highly potent opportunistic pathogen, due to its ability to acquire invasive resistance against antimicrobial chemotherapies. *S. aureus* results in array of infections from a carbuncle, furuncle, abscesses and sepsis to life threatening bacteremia, endocarditis and osteomyelitis. Prevalence of MRSA (Methicillin resistant *Staphylococcus aureus*) and VRSA (Vancomycin resistant *Staphylococcus aureus*) exhibits high morbidity and mortality rates globally in hospital and community acquired infections. Scenario of antibiotic resistance has changed considerably after the 1970 [1]. According to Center for Disease Control and Prevention (CDC), United States, approximately 2 million cases of acquired nosocomial infections [2]. Nearly 94,360 MRSA infections (invasive) and approximately 18,650 deaths are reported annually [3]. Anti MRSA treatment costs around over three times the monthly income of more than 40% of the Indian population [4]. Antibiotic resistance patterns vary geographically. Molecular typing methods turned out to be very fruitful in investigating the local and global epidemiology and outbreak of nosocomial infections [5]. Availability and utilization of particular agents in a given environment, determine the rate of resistance within microbial populations [6]. Advanced antimicrobial agents such as linezolid, daptomycin, tigecycline and quinupristin/dalfopristin are used in the treatment of MRSA infections. Even though combination treatment options resolve to some extent, but resistance against modern chemotherapies pose a challenge to the medical practices. Second-line drugs like glycopeptides, dalbavancin, telavancin and otritavancin are the current choice of drugs for the treatment for VRSA infections. However, they are more expensive and causes severe side effects, hence monitoring during treatment is advisable, which increases the cost even further [7].

In India prevalence of *S. aureus* infections are poorly studied, very few reports are available to monitor the emergence of MDR (Multi drug Resistant) strains of *S. aureus*. The prevalence of antibiotic resistance patterns has been studied in developed countries, but in lower middle income countries like India occurrence and treatment cost caused by MDR strains are poorly studied. Reports on the incidence of MRSA and VRSA are sparsely available for different geographical areas of India [8–17].

The present study reports the prevalence of MRSA and VRSA and antibiotic resistance patterns among the 212 *S. aureus* isolates

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obtained from different cities across India and the antibiotic susceptibility test performed against 14 different classes of antibiotics used to treat various types of infections caused by *S. aureus*.

2. Materials and methods

2.1. Sample collection

A total of 387 clinical specimens such as pus, blood and urine were collected from diagnostic and health care centers from different cities in India, namely, Delhi, Bangalore, Palakkad, Chennai and Gulbarga via., transport media. All the samples were enriched with brain heart infusion broth and incubated overnight at 37 °C.

2.2. Identification and characterization of isolates

The isolates were identified on the basis of cultural, morphological characteristics on mannitol salt agar, blood agar, Baird-Parker agar and biochemical tests like coagulase and Dnase carried out as per standard protocols [18]. Standard *S. aureus* isolates ATCC43300 and MTCC 96 were included in the study for comparison.

2.3. Antimicrobial susceptibility testing

Following antimicrobial discs (Hi media, India) were used in the study: penicillin (P)- 10units, oxacillin (OX)- 1mcg, cefoxitin (CX) - 30mcg, amoxyclav (amoxycillin/calvulanic acid) (AMC) –20mcg, cefotaxime(CTX) –30mcg, ceftazidime(CAZ) - 30mcg, cefpodoxime(CPD) - 10mcg, vancomycin (VA)-30mcg, amikacin(AK) - 30mcg, tobramycin (TOB) - 10mcg, tetracycline (TE) - 30mcg, doxycycline (DO) - 30mcg, ciprofloxacin (CIP) - 5mcg, ofloxacin(OF) - 5mcg, clindamycin(CD) - 2mcg, clotrimoxazole(COT) (trimethoprin-sulfamelthoxazole) 25mcg (1.25/23.75mcg), chloramphenicol(C) - 30mcg, rifampicin (RIF) - 5mcg, erythromycin(E) - 15mcg, linezolid(LZ) - 30mcg. Antimicrobial susceptibility and MIC for vancomycin and oxacillin were performed as per CLSI (2014) guidelines [19]. The isolates were tested for the presence of induced clindamycin resistance according to CLSI criteria. Isolates were resistant to erythromycin and sensitive to clindamycin were tested for inducible resistance by the D-test. Erythromycin and clindamycin discs were placed 15 mm apart on Mueller Hinton agar plate, incubated at 37 °C for 24 h, D-test positivity was identified by flattening of the clindamycin zone between the erythromycin and clindamycin discs [19].

3. Results

Out of the 387 clinical samples collected from different cities across the country, 212 (54%) were positive for the isolation of *S. aureus* (Table 1). Maximum number of isolates (120) were obtained from pus samples, followed by urine (47) and blood (45) respectively. Similarly, maximum number of isolates has been obtained from the samples collected from Delhi (82) and from other centers the number ranged from 26 to 39.

The antibiogram pattern of the 212 isolates determined using 21 antibiotics belonging to 14 classes is shown in Fig. 1. High percentage resistance has been observed against pencillinase labile penicillins. The resistance observed against macrolides, fluoroquinolones and cephems is also on the higher side. Resistance to the other classes of antibiotics was moderate, however the resistance was comparatively low against the phenicols and glycopeptides.

About 45% of the *S. aureus* isolates MRSA which include 55.31% from urine, 46.66% from blood and 40% from pus (Fig. 2). The incidence of MRSA was comparatively high in the isolates from Bangalore (57.14%) and Palakkad (56.41%) while the lowest incidence was found in the isolates from Delhi (34.14%) (Fig. 3).

Similarly, out of the 212 isolates of *S. aureus*, 10.84% were found to be VRSA. Incidence of VRSA was maximum in the Urine samples (15.55%) followed by blood (10.63%) and lowest was in pus samples (9.16%) (Fig.4). The distribution of VRSA in different cities is shown in Fig. 5, which shows that maximum VRSA are from samples collected from Palakkad (20.51%) and lowest in the samples from Chennai (3.3%).

Agar dilution method was adapted to determine the MIC for oxacillin (MRSA) and vancomycin (VRSA) among the *S. aureus* isolated from different cities in India [22]. The MRSA strains were further confirmed by determining the MIC of isolates against oxacillin. MIC 8mcg/ml confirms MRSA. 95 isolates showed the MIC of 8 mcg/ml or more indicating MRSA incidence of 44.81%. Vancomycin MIC ranged from a minimum of 8 mcg and to a maximum of 70 mcg/ml (Fig. 6). It is alarming that as many as 7 isolates showed a very high MIC of 70 mcg/ml.

All the MRSA were tested for inducible clindamycin resistance and almost 70% of MRSA are showing positive results to D-test (Fig. 7), which is confirmatory for inducible clindamycin resistance among erythromycin resistant MRSA. A few MRSA and VRSA isolates have been found to be susceptible to tigecycline and quinupristin/dalfopristin.

Table 1
The number of *S. aureus* isolated from different centers.

Sample	Different cities of India					Total
	Bangalore	Chennai	Delhi	Palakkad	Gulbarga	
Pus	27	8	55	9	21	120
Blood	5	7	19	12	2	45
Urine	3	15	8	18	3	47
Total	35	30	82	39	26	212

4. Discussion

Multi drug resistant forms of *Staphylococcus aureus* strains are a serious concern worldwide. Emergence of MDR strains against advanced antibiotics is a major drawback of chemotherapy. In the

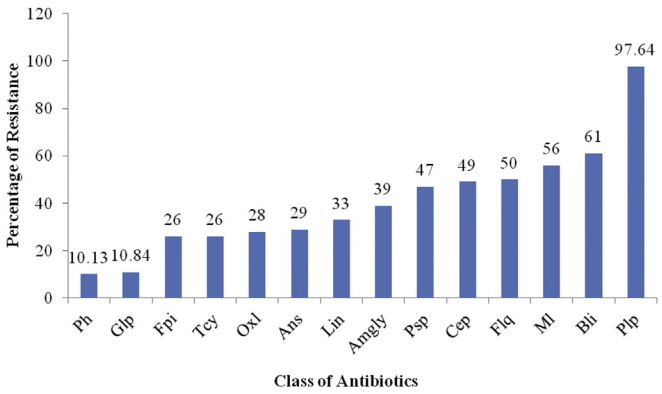

Fig. 1. Antibiotic resistance patterns of the *S. aureus* isolates. (Ph = Phenicols, Glp = Glycopeptides,Fpi = Folate pathway inhibitors, Tcy = Tetracyclines, Oxl = Oxazolidinones, Ans = Ansamycins, Lin = Lincosamides, Amgly = Aminoglycosides, Psp = Penicillinase stable penicillins, Cep = Cephems, Flq = Floroquinolones, Ml = Macrolides, Bli = β-Lactam/β-lactamase inhibitor combinations, Plp = penicillinase laible Penicillins).