

Plasmid Analysis and Prevalence of Multidrug Resistant *Staphylococcus aureus* Reservoirs in Chennai City, India

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Citation

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Abstract

The present study was designed to investigate the prevalence of multidrug resistant *Staphylococcus aureus* carriers in Chennai, India. A total of 40 clinical samples comprising of 20 urine and 20 serum samples, collected from healthy individuals of both sex between the age group of 25-50 years in Chennai city were used in this study. About 57.5 % of samples were positive for *S. aureus* and all of them exhibited β -haemolysis on blood agar. All the isolates were resistant to methicillin, bacitracin, novobiocin and sensitive to norfloxacin. None of the isolates were susceptible to all the antibiotics tested. The MAR index of the isolates ranged between 0.18 and 0.63. All the isolates harboured a 23 kb plasmid.

INTRODUCTION

Staphylococcus aureus is one of the major human pathogens widely spread in the environment. It has persisted as an important hospital and community pathogen. They are responsible for more than 80 % of the supportive diseases encountered in medical practice (Kalsoom and Abdul, 2006). This organism has been found to be the most common bacterial agent recovered from blood stream infections, skin and soft tissue infections and hospital-acquired post-operative wound infections (Giacometti et al., 2000; Doern et al., 1999). It is also associated with a variety of clinical infections including septicemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis with substantial rates of morbidity and mortality (Shopsin and Kreiswirth, 2001; Engemann et al., 2003; Cosgrove et al., 2003). About 30-50% of the human populations are *Staphylococcus* carriers (Bhatia and Zahoor, 2007). These bacteria can survive on dry surfaces increasing the chances of transmission. *S. aureus* infections can spread through contact with pus from an infected wound, skin to skin contact with infected person and contact with objects used by infected person.

Antimicrobial resistance has been noticed as one of the paramount microbial threats of the twenty first century (Smolinski et al., 2003). *S. aureus* has been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by

the emergence of resistant forms of pathogens (Alborzi et al., 2000). Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a challenge for public health; as community associated infections appear to be on the increase in both adults and children in various regions and countries (Layton et al., 1995).

The present study reports the prevalence, antimicrobial resistance and the plasmid profile of *S. aureus* isolates from serum & urine samples of healthy individuals in Chennai, India.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 40 clinical samples comprising of 20 urine and 20 serum samples, collected during a period of three months between December 2007 and February 2008 from healthy individuals of both sex (male & female) in Chennai city were screened for the presence of *S. aureus*. All samples were collected from healthy individuals between the age group 25-50 years who came to clinical lab for testing occupational diseases. Notably, they all were not suffering from any infections caused by *S. aureus*. Equal numbers of samples were collected from both the sex. Neither of the two samples (urine & serum) was collected from the same individual.

BACTERIAL ISOLATION

All the samples were collected in sterile containers & processed aseptically. All the samples were examined individually for the existence of *S. aureus* by plating them on Mannitol salt agar (HiMedia) and incubated at 37 °C for about 24 hr. The characteristic colonies were aseptically isolated and the bacterial strains were subcultured on nutrient agar slants and stored at 4°C for future use.

The isolated strains were identified to their species level by Gram staining and standard biochemical tests such as catalase, urease, oxidase, citrate utilization, indole, methyl red and Voges Proskauer test. Identification of isolates was confirmed by direct-tube coagulase test with plasma.

HAEMOLYTIC ACTIVITY

The haemolytic activity of the *S. aureus* isolates were determined by blood agar plate assay (Breneder and Janda, 1987). Pattern of haemolysis around the colonies on blood agar plates containing 5 % (v/v) blood were recorded after 24 hr incubation at 37 °C.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility of the *S. aureus* isolates was determined by disc diffusion method (Bauer and Kirby, 1966) against 11 commercially available antibiotic discs (HiMedia). The isolates were enriched in Brain Heart Infusion Broth (BHIB, HiMedia) for 8 hr. The enriched cultures were then swabbed on Mueller Hinton Agar (HiMedia) plates using sterile cotton swabs. The discs were placed on the agar surface. After 24 hr incubation, the diameter of the inhibition zones was recorded and the resistance data was tabulated. The resistance pattern was determined using zone size interpretative chart (HiMedia), which was in accordance to the performance standards for Antibiotic susceptibility testing, CLSI.

MAR INDEX

Multiple Antibiotic Resistance (MAR) index value of all the strains were calculated. The MAR index applied to a single isolate is defined as a/b , where 'a' represents the number of antibiotics to which the isolate was resistant and 'b' represents the number of antibiotics to which the isolate was subjected (Krumperman, 1985).

PLASMID DNA ISOLATION

All the isolates were subjected to plasmid DNA extraction by alkali lysis method as described by Bimboim, 1983. The extracted plasmid DNA was electrophoresed on 1.2% agarose gel stained with ethidium bromide. About 20 µL of

plasmid DNA preparation were loaded into each well and electrophoresed at 50 volts for 1 hr. Plasmid DNA band was observed with UV transilluminator and photographed. Molecular weight of the DNA bands was calculated by comparing with Lambda DNA Hind III digest (Banglore genei) as standard marker.

RESULTS

Among the 40 samples analyzed, 57.5 % (23/40) of the samples were positive for fermentation of Mannitol Salt Agar. All the isolates were identified as Gram positive cocci in clusters under microscopic examination. The isolates were positive for methyl red, Voges Proskauer, catalase, and coagulase test. The biochemical characteristics of the isolates from clinical samples are indicated in Table 1. All the isolates showed α -haemolytic pattern on blood agar.

Figure 1

Table 1: Biochemical characteristics of the isolates from serum and urine samples collected among healthy individuals

S.No	Biochemical characteristics	% of isolates positive
1	Indole	4.17
2	Methyl Red	100
3	Voges-Proskauer	91.66
4	Citrate	16.67
5	Catalase	100
6	Oxidase	20.83
7	Urease	100
8	coagulase test	100

ANTIBIOTIC RESISTANCE

The antibiotic resistance data of *S. aureus* isolates are shown in Table 2. The isolates were resistant to methicillin (100%), amoxicillin (91.3%), bacitracin (73.91) and novobiocin (86.95%). The isolates were sensitive to norfloxacin, gentamicin, kanamycin, tetracycline, vancomycin and nitrofurantoin. None of the isolates were susceptible to all the tested antibiotics.

Figure 2

Table 2: The antibiotic resistance data of isolates collected among healthy individuals

Antibiotics	No. of Urine isolates resistant to antibiotics (n = 12)	% isolates resistant in urine samples	No. of Serum isolates resistant to antibiotics (n = 11)	% isolates resistant in serum samples	% Total isolates resistant to antibiotics (n = 23)
Amoxicillin	10	83.33	11	100	91.3
Erythromycin	4	33.33	2	18.18	26.08
Gentamicin	1	8.33	-	-	4.34
Methicillin	12	100	11	100	100
Kanamycin	-	-	2	18.18	8.69
Tetracycline	1	8.33	1	9.09	8.69
Vancomycin	-	-	1	9.09	4.34
Bacitracin	8	66.66	9	81.81	73.91
Nitrofurantoin	1	8.33	-	-	4.34
Norfloxacin	-	-	-	-	-
Novobiocin	10	83.33	10	90.9	86.95

MAR INDEX

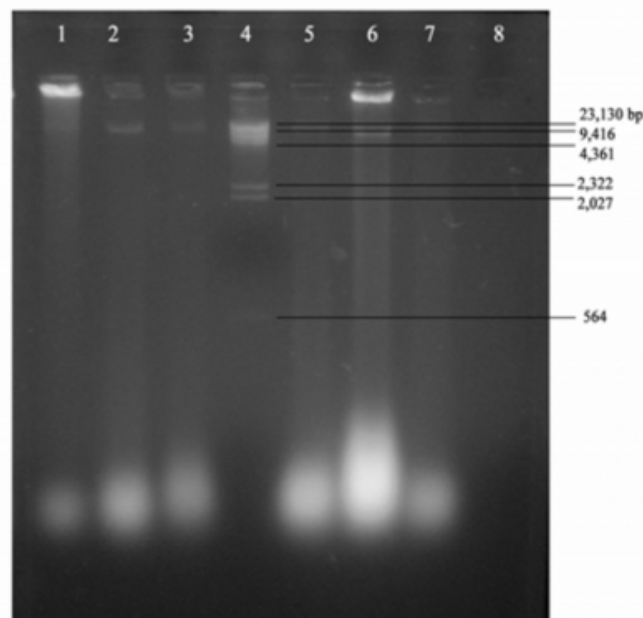
All the isolates were multiple antibiotic resistant. The MAR index value ranged between 0.18 and 0.63. Notably, 39.13% of isolates were resistant to 4 / 11 (MAR index – 0.36) antibiotics tested. Among the isolates, 21.73% and 17.39 % of them were resistant to 3 / 11 (MAR index – 0.27) and 5 / 11 (MAR index – 0.45) antibiotics tested respectively.

PLASMID DNA ANALYSIS

The plasmid DNA analysis of the isolates showed the presence of one plasmid in all the isolates. A 23 kb plasmid DNA was observed in all the isolates when compared with the Lambda DNA Hind III digest (Bangalore genei, India) as shown in Fig. 1.

Figure 3

Fig.1. Plasmid profile of isolated from healthy carriers in Chennai city. Lane 1-3 (Serum isolates), 5-7 (Urine isolates) represents isolates harboring 23 kb plasmid. Lane 4 represents Lambda DNA digest. Lane 8 empty.

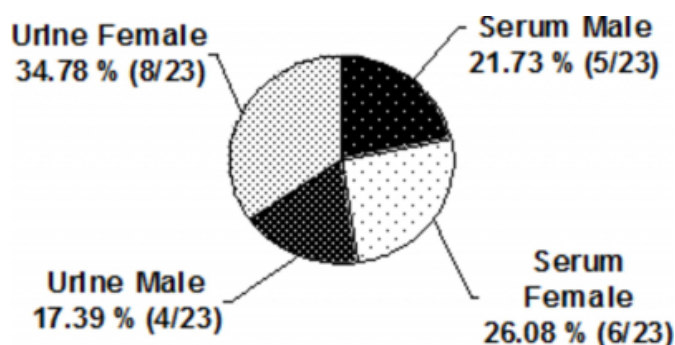


DISCUSSION

This study is the first report on the prevalence of multiple antibiotic resistant *S. aureus* isolated from healthy individuals in Chennai city, India. Notably, all the samples were collected from healthy individuals with no distinct symptoms observed in them for any of the diseases caused by the pathogen of our interest. Among the samples tested, 55 % of serum samples and 60 % of urine samples were positive for *S. aureus*. It was also observed that higher prevalence of *S. aureus* was recorded in female urine samples (34.78%) (Fig.2). This data is in agreement with Onanuga et al. (2005) who reported 36 % incidence of *S. aureus* in the urine samples tested among the healthy women.

Figure 4

Fig.2. Prevalence of in serum and urine samples collected among healthy male & female respectively



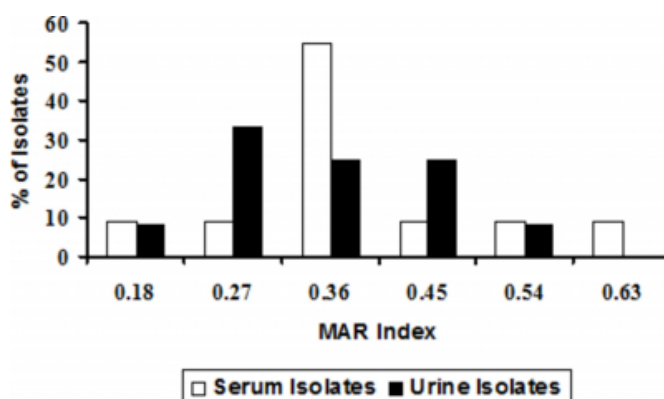
Higher prevalence of *S. aureus* (57.5 %) in healthy individuals was observed in the present study, compared to results recorded from clinical samples by several workers. Anbumani et al. (2006) reported 38 % incidence of MRSA from the blood cultures in Chennai. The results of this study is also in contrary to the results recorded by Rajadurai pandi et al. (2006) who reported only 28.4 % and 14 % incidences of *S. aureus* respectively from the blood and urine samples collected from major southern districts of Tamil Nadu.

All the isolates were resistant against amoxicillin, methicillin, bacitracin and novobiocin. The isolates showed least antibiotic resistance against other antibiotics tested. Onanuga et al. (2005) reported 16 % and 89 % of the isolates from healthy woman were resistant to gentamicin and vancomycin respectively which is in contrary to the present study. Only one isolate from serum sample showed resistance to vancomycin. Rajadurai pandi et al. (2006) and Anbumani et al. (2006) reported none of the isolates were resistant to vancomycin. This variation in the drug resistance may be well related to the type of antimicrobial agents prescribed for treating various diseases in different geographical areas (Radu et al., 1997).

The MAR index value ranges between 0.18 and 0.63. All the isolates showed resistance to atleast 2 of 11 antibiotics tested. The incidence of *S. aureus* isolates based on MAR index was shown in figure 3.

Figure 5

Fig. 3. Percentage prevalence of in serum and urine samples based on MAR index



Several workers have reported the multiple antibiotic resistance patterns of *S. aureus* from clinical samples (Assadullah et al., 2003; Anupurba et al., 2003; Krishna et al., 2004). when low doses of antibiotics are used against bacteria, they inhibit the growth of susceptible bacteria, leaving the smaller number of already resistant bacteria to thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells then eventually affecting other cells (Craig, 1998).

The plasmid analysis of the isolates revealed the presence of 23kb plasmid in all the isolates. Resistance in MRSA is also related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin binding protein called PBP2a. Multiple drug resistance of *S. aureus* is due to several drug resistant genes in a single plasmid, each with its own resistance markers. Rahman et al. (2005) reported the presence of 23 kb plasmid in *S. aureus* strains isolated from skin lesion sample which is in agreement with the present study (figure 1). The results of Rahman et al. (2005) confirmed that the 23 kb plasmid isolated from *S. aureus* might be carrying the genes coding for multidrug resistance and the plasmids were transferable.

The results of the present study are in agreement to the postulation that healthy members are the highest reservoir of multidrug resistant bacteria (Lamikanra et al., 1996). However, this study involves only a small number of isolates. So, a multi centre study should be well planned and done to determine the prevalence of multidrug resistant *S. aureus* reservoirs in the society. It is also necessary to develop policies to restrict the use of antibiotics and also to establish monitoring systems for rapid identification of epidemics.

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