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ORAL CARRIAGE AND ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* IN DENTAL HOSPITAL STAFF AND HEALTHY GENERAL POPULATION IN KOLKATA, INDIA

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ABSTRACT

The oral cavity carriage and antibiotic susceptibility patterns of *Staphylococcus aureus* in Dental hospital staff and healthy general population were determined. Oral cavity swabs were taken from 113 healthy general population and 90 health care workers. Antibiotic disc susceptibility testing was conducted following the CLSI method. *Staphylococcus aureus* carriage was noted in 28.3% of healthy general population and 38.9% of health care workers. Resistance to commonly used oral antibiotics of healthy general population & health care workers, ampicillin 93.8% & 97.2%, amoxicillin/clavulanic acid 84.4% & 77.2%, amoxicillin 43.8% & 57.2%, ciprofloxacin 53.2% & 57.2% and ofloxacin 37.5% & 42.9%, respectively. 5.7% methicillin resistant *Staphylococcus aureus* was detected among the hospital personnel from isolated strain. The MRSA isolates showed multiple drug resistance (MDR), except imipenem. Hospitals should assess the advantages and disadvantages of routinely culturing personnel, however, in outbreak situation hospital personnel especially young persons may be sources of nosocomial infection.

KEY WORDS: *Staphylococcus aureus*, oral colonization, healthcare workers

Staphylococcus aureus is a common human pathogen that causes various skin and mucosal infections. Beside superficial infections the organism also causes abscess formation, septicaemia, pneumonia, osteomyelitis, meningitis, pneumonia and toxic shock syndrome (Cheesbrough, 2002 and Talaro, 20020). *S. aureus* is a Gram positive, nonmotile, catalase positive, coagulase positive, facultative anaerobe and it is considered as the most resistant of all non-spore forming pathogens, with well developed capacities to withstand high salt (7.5-10%), extremes in pH and high temperature (up to 60°C for 60 minutes). It also remains viable after months of air-drying and resists the effects of many disinfectants and antibiotics (Talaro, 2002). Nosocomial infections of which *Staph. aureus* is a typical example, are known to account for morbidity and mortality of millions of patients annually worldwide (Mansouri et al., 199). *S. aureus* is frequently found on normal human skin and mucous membranes. Although the oral cavity harbors a complex microflora consisting of mostly non-pathogenic microorganisms, it was of interest to investigate the occurrence of *S. aureus*. There are several reports of the isolation of *S. aureus* as a component of the resident oral flora from human community.

S. aureus is known to be notorious in their acquisition of resistance to new drugs and continues to defy attempts at medical control (Talaro, 2002). Many strains of *S. aureus* carry a wide variety of multi-drug resistant genes on plasmids.The resistance of *S. aureus* isolates from different parts of the world to commonly used antibiotics has been widely reported. Historically antibiotic-resistant strains of *S. aureus* were first identified in 1942, just after the beginning of clinical treatments with penicillin (Owen, 1994). In the late fifties, semi-synthetic penicillins, like methicillin, were developed to solve this problem, but only two years later, methicillin resistance was reported (Suzuki et al., 1997). Methicillin and Oxacillin are similar antibiotics; MRSA is the usually accepted designation. Generally once MRSA appears in an institution; it becomes established as persistent cause of nosocomial infection. However, MRSA strains have spread in many hospitals isolates worldwide since 1970s (Hiramatsu et al., 2001). Hospital personnel tend to have higher colonization rates than the general population. Now this problem seems to be moving beyond the hospital environment. Recent reports showed that the number of community-acquired MRSA, infections had increased (Moreno et al., 1995) and this is an alarming problem throughout the world. The occurrence of MRSA in the nostril, skin wounds and respiratory tract has been well documented in India and rest of the world, but little is known about its presence in the oral cavity or the potential implications for the practice of dentistry and

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practically no study has been done so far in Indian community.

Despite of this fact there is still no unique protocol for finding and controlling *S. aureus* infection in Dental hospital. Thus, we designed this study to ascertain the rate of oral carriage of *S. aureus* in the hospital personnel and general population.

METHODS

This was prospective study conducted during 12 months from March, 2011 to February 2012. The study was conducted in Gurunanak Institute of Dental Science and Research; Panihati, North 24 Parganas, Kolkata, West Bengal, India.

Hospital personnel are including in Doctor (Medical and Dental), Medical and Dental technologist, hospital cleaners/ helpers and general population are including office staff, non-medical faculty, other technical staffs, students, medical/sales representative, relatives or friends of the patient and staff. Having explained our goal and requested them to fill an informed consent, hospital personnel and general population were evaluated for *Staphylococcus aureus* oral colonization. Initial data including name, sex, age, past medical history, oral habit (smoking or others), use of mouth wash etc. were gathered by a questionnaire. Past medical history of underlying diseases that might increase the chance of colonization, such as, chronic renal disease, insulin dependent diabetes mellitus, and dialysis were inquired (Massachusetts Department of Public Health, 1997). None of the subjects had received antibiotic for at least one week prior to the study. In case of Health care workers at least two years working criteria are being observed and following on.

Collection and Processing of Samples

Oral cavity swabs are collected from Health care workers (HCWs) and General populations with no oral complaints, using sterile swab sticks. Oral cavity swabs were taken from 113 healthy general population and 90 hospital personnel had no definite oral or dental complaints. The samples were cultured aerobically in Mannitol salt agar media (Himedia Laboratories Pvt. Ltd.; Mumbai). The plates were incubated aerobically at 37°C for 24 hrs. Streak plate technique was used to obtain pure culture of each isolate prior to identification.

Identification of Isolates

The isolates were identified using colony morphology with Mannitol fermentation by colour change of the medium around each colony from red to yellow (used of Mannitol salt agar), Gram staining, Catalase test, Coagulase test (slide and tube method) and DNase test as described by Cheesbrough, 2002. Sensitivity testing using Kirby-Bauer disc diffusion technique (Bauer et al. 1966)). The following concentration of antibiotic per disc as recommended by Clinical Laboratory Standards Institute (CLSI,2007) (Himedia Laboratories Pvt. Ltd.; Mumbai) , Amoxicillin (20 mcg), Amoxycillin + Clavulanic acid (20+10 mcg), Ampicillin (10 mcg), Ampicillin + Sulbactam (10+10 mcg), Cefpodoxime(10 mcg), Ciprofloxacin (5 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), Rifampicin (5 mcg), Imipenem (10 mcg), Linezolid (30 mcg), Ofloxacin (5 mcg),Piperacillin (100 mcg), Piperacillin + Tazobactam (100+10 mcg), Ticarcillin (75 mcg), Ticacillin + Clavulanic acid (75+10 mcg), Meropenem (10 mcg), Vancomycin (30 mcg), Oxacillin (1 mcg).

Resistance or Susceptibility was reported based on the CLSI guideline. Two hours Tryptone Soya Broth (Himedia; Mumbai) (3ml) cultures at 37°C of each isolate were adjusted to McFarland turbidity (0.5), and the disc sensitivity screening conducted as described by Cheesbrough, 2002. Sterile swabs were used to inoculate the test organism onto the sensitivity agar (Mueller Hinton agar media) (Himedia; Mumbai). Sterile forceps were used to carefully distribute the antibiotic discs evenly on the inoculated plates. After allowing for about 30 minutes on the bench for proper diffusion, the plates were inverted and incubated aerobically at 35°C for 18 hours. The inhibition zone diameters were measured in millimeters using meter rule.

Methicillin Resistant *S. aureus* detection (MRSA)

Methicillin-resistance was verified by the CLSI (formerly NCCLS) Oxacillin screening test (NCCLS, 2000). Oxacillin sensitivity was performed on Mueller Hinton agar media with 4% sodium chloride. The strains

were reported as sensitive, or resistant, to Oxacillin (1 mcg) with inhibition zone diameter equal or more than 13 mm and less than or 10 mm respectively. Disk diffusion testing was performed as recommended by the National Committee for Clinical Standards; briefly, a broth culture suspension of the isolate to be tested was prepared in Trypticase soya broth and turbidity adjusted to a 0.5 McFarland standard. The zone sizes were read after 24 hours of incubation in ambient air at 35°C. Isolates were classified as either susceptible Bauer et al.,(1966). American Typing Collection (ATCC 25923) of *S. aureus* was used as a control strain in antibacterial susceptibility testing.

RESULTS

A total of 35 of 90 (38.9%) health care workers were *Staphylococcus aureus* carriers compared to 32 of 113 (28.3%) of the healthy general population (Table, 1 and 2).

Antibiotic Susceptibility Patterns

Antibiotic disc susceptibility testing was carried out on all the 67 *Staphylococcus aureus* isolates. The strains isolated from two sites from the same subject if the strains exhibited the same antibiotic susceptibility pattern. Strains that exhibited different susceptibility patterns even though isolated from the same subject will be analyzed as separate strains (Table ,3).

Out of the 32 strains isolated from the healthy general population, a low percentage of these strains were resistant to ampicillin/sulbactam & rifampicin (6.3%), linezolid & piperacillin/tazobactam (9.4%), meropenem (15.7%), ofloxacin (37.5%), ticarcillin/clavulanic acid (40.7%), amoxycillin (43.8%), ciprofloxacin (53.2%), piperacillin (56.3%), vancomycin (56.8%), clindamycin & ticarcillin (68.8%), amoxycillin/clavulanic acid (84.4%), cefpodoxime (87.5%), erythromycin (90.7%) and ampicillin (93.8%). All strains were sensitive to oxacillin & imipenem.

Out of the 35 strains isolated from the health care workers, a low percentage of the strains were also resistant to oxacillin (5.7%), rifampicin (8.6%), linezolid (14.3%), ampicillin/sulbactam (17.2%), piperacillin/tazobactam (20.0%), meropenem (22.9%), clindamycin (34.3%), ofloxacin (42.9%), amoxycillin, ciprofloxacin & ticarcillin/clavulanic acid (57.2%), piperacillin (60.0%), ticarcillin & vancomycin (71.5%), amoxycillin/clavulanic acid (77.2%), erythromycin (82.9%), cefpodoxime (85.8%)

Table: 1

Distribution of oral carrier of *Staph. aureus* according to different age group and sex of healthy general population of subjects:

Age group (Years)	Male				Female			
	Total Samples	Total Isolated	MRSA	MSSA	Total Samples	Total Isolated	MRSA	MSSA
0-10	02	01	00	01	02	00	00	00
11-20	02	02	00	02	02	01	00	01
21-30	20	07	00	07	12	03	00	03
31-40	26	08	00	08	12	02	00	02
41-50	15	03	00	03	05	02	00	02
51-60	05	01	00	01	03	01	00	01
61and above	04	01	00	01	03	00	00	00
Total	74	23	00	23	39	09	00	09
Percentage (%)		31.0	00	31.0		23.0	00	23.0

Table: 2

Distribution of oral carriers of *Staph. aureus* according to different groups and sex of health care workers (HCWs) of subjects:

Group	Mean age (years)	Male				Female			
		Total Samples	Total Isolated	MRSA	MSSA	Total Samples	Total Isolated	MRSA	MSSA
Doctor (Medical / Dental)	30.6	14	03	01	02	09	01	00	01
Medical / Dental Technologist	33.0	30	13	01	12	10	02	00	02
Cleaners / Helpers	27.0	18	11	00	11	09	05	00	05
Total		62	27	02	25	28	08	00	08
Percentage (%)			43.5	3.2	40.3		28.5	00	28.5

*MRSA: Methicillin-resistant *Staph. aureus*.

*MSSA: Methicillin-sensitive *Staph. aureus*.

and ampicillin (97.2%). All strains were sensitive to imipenem. The MRSA isolates showed multiple drug resistance (MDR), except imipenem.

DISCUSSION

Staphylococcus aureus carriage appears to play a key role in the epidemiology and pathogenesis of infections because carriage often precedes infection . Healthy individuals have a slight risk of invasive infection caused by *S. aureus* during their carrier state but they can be carriers of the organism, because its primary habitat is moist squamous epithelium of the anterior nares and oral cavity. *S. aureus* nasal carrier rate of 20-50% can be found in normal adults (Hizeh et al., 1997). *S. aureus* carriage differs from one individual to another. Persistent or transient carriers among the hospital personnel may disseminate *S. aureus* into the hospital environment. Haley et al., (1982) reported an outbreak due to direct dissemination from colonized personnel. Studies from Turkey showed different hospital personnel *S. aureus* carrier rates, ranging 15.3-31.5 % (Hizeh et al. 1997). Also Verghese et al. ,(1999) found 18.2% of health care workers to be nasal carriers, of these, 12.2% were carriers of MRSA. In our study, 28.3% of the healthy general population carries *S. aureus* while 38.9% of the hospital personnel were oral carriers. In the healthy general population, the rate of *S. aureus* in males and females were 31.0% and 23.0%, respectively. The rate of *S. aureus* among the hospital personnel, male and female were 43.5% & 28.5% respectively. The mild difference of carrier rate between male and female can be explained by lower personal hygiene and higher tobacco habit in male than female. The carriage rate in this healthy general population is in concordance with other studies in healthy communities. Carriage rates in the health care workers in this study were higher than in the healthy general population. Several assumptions can be made as to why the rate is higher among these hospital personnel. It could be due to the personal hygiene of these hospital personnel, as they could not take care of themselves. The close proximity to other hospital personnel could possibly lead to easy transmission of the strains among themselves and also spread of the bacteria from one hospital personnel to another by the health care workers in the facility.

Table: 3
Percentage susceptibility of isolated *Staphylococcus aureus* to tested antibiotic

	Health care workers (Total Isolated :35)				General population (Total Isolated :32)			
Antibiotics	**S(No.)**	**R(No.)**	**%S**	**%R**	**S(No.)**	**R(No.)**	**%S**	**%R**
Amoxycillin	15	20	42.8	57.2	18	14	56.2	43.8
Amoxycillin/clavulanic acid	08	27	22.8	77.2	05	27	15.6	84.4
Ampicillin	01	34	2.8	97.2	02	30	6.2	93.8
Ampicillin/sulbactam	29	06	82.8	17.2	30	2	93.7	6.3
Cefpodoxime	05	30	14.2	85.8	04	28	12.5	87.5
Ciprofloxacin	15	20	42.8	57.2	15	17	46.8	53.2
Clindamycin	23	12	65.7	34.3	10	22	31.2	68.8
Erythromycin	06	29	17.1	82.9	03	29	9.3	90.7
Meropenem	27	08	77.1	22.9	27	05	84.3	15.7
Imipenem	35	00	100	00	32	00	100	00
Linezolid	30	05	85.7	14.3	29	03	90.6	9.4
Ofloxacin	20	15	57.1	42.9	20	12	62.5	37.5
Oxacillin	**33**	**02**	**94.3**	**5.7**	**32**	**00**	**100**	**00**
Piperacillin	14	21	40.0	60.0	14	18	43.7	56.3
Piperacillin/Tazobactam	28	07	80.0	20.0	29	03	90.6	9.4
Ticarcillin	10	25	28.5	71.5	10	22	31.2	68.8
Ticarcillin/clavulanic acid	15	20	42.8	57.2	19	13	59.3	40.7
Vancomycin	10	25	28.5	71.5	14	18	43.2	56.8
Rifampicin	32	03	91.4	8.6	30	02	93.7	6.3

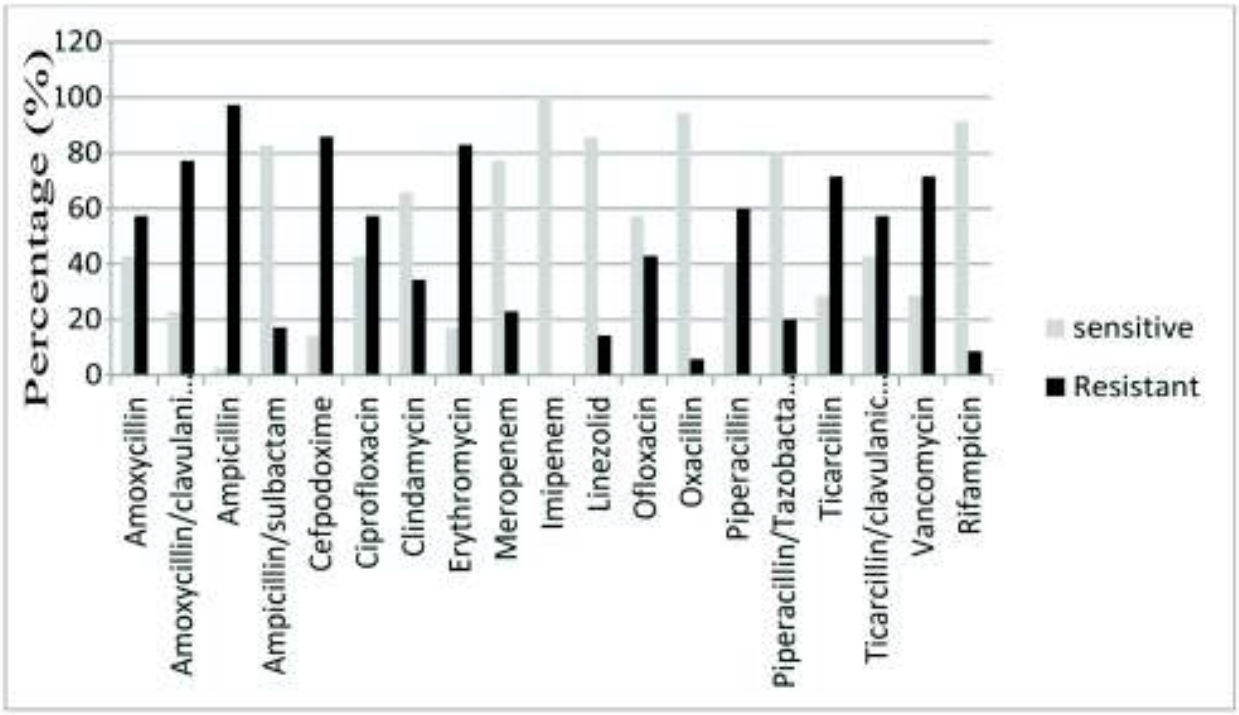

Figure 1: Pattern of *Staphylococcus aureus* susceptibility (Healthcare workers)

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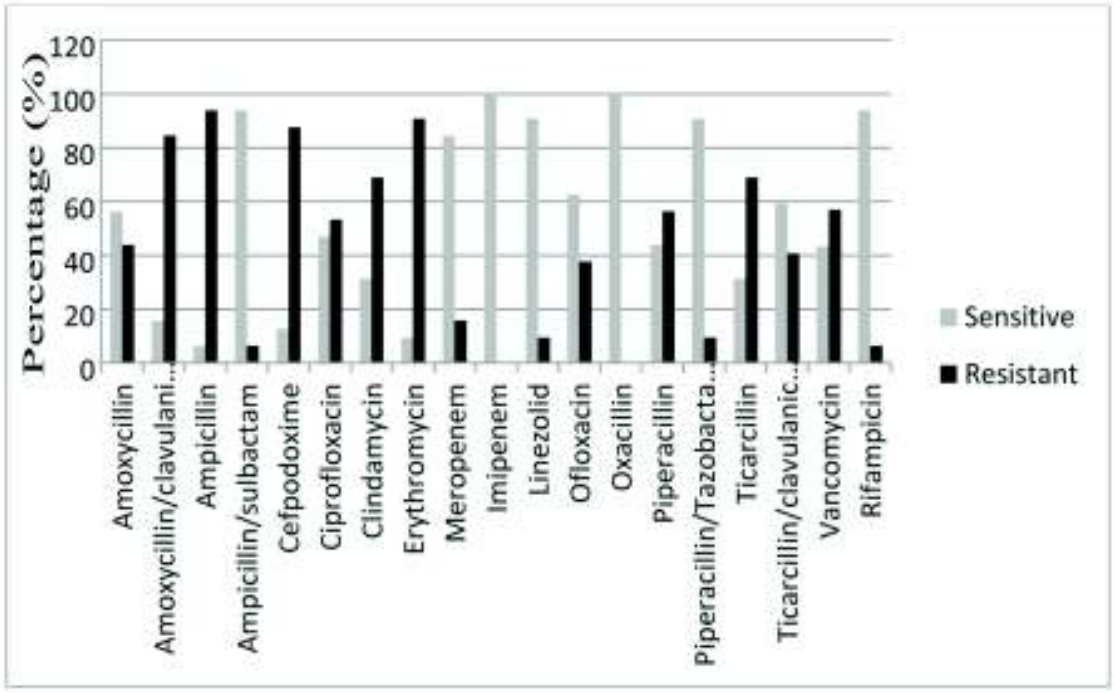

Figure 2: Pattern of *Staphylococcus aureus* susceptibility (General population)

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In this study, colonization with multiple strains was mainly observed more frequently in the healthy general population as compared to the health care workers. Zimakoff et al., (1996) reported that staphylococcal infection occurred significantly more frequently among carriers, and more than half of the patients were infected by the same or possibly the same strain as they carried in the nose, oral cavity or on skin.

MRSA has been detected in nursing and long term care facilities in different countries. MRSA persisted in the oral cavities of children for more than five years with the potential to cause nosocomial infections (Wagenvoort et al., 2000). MRSA colonization has been found in 2% of the hospital personnel in various studies (Hizeh et al., 1997). Healthcare providers may become transient or persistent MRSA carriers whilst working in hospitals in which MRSA is endemic. They may then become a source of infection for patients as well as their own families. In this study, 5.7% MRSA was detected among the hospital personnel from isolated *S. aureus*. The rate of MRSA among the hospital personnel, male and female were 3.2% & 0.0% respectively shown in Table,2. Multi-antibiotic resistance *S. aureus* strains are also found in our studies. Data from Table,3, Figure,1 and Figure,2 show that majority of isolated *S. aureus* strain from health care workers and general population are resistant to commonly used oral antibiotics such as ampicillin, amoxycillin/clavulanic acid, amoxycillin, ciprofloxacin, ofloxacin. The MRSA isolates showed multiple drug resistance (MDR), except imipenem. It is known that routine screening of hospital personnel for *S. aureus* colonization requires considerable time expenditure, and if colonized personnel are removed from patient care, routine services of that hospital will be disrupt. Thus, periodic screening for the hospital personnel with a carrier rate about 15% is not recommended. Personnel should be cultured when they are thought to be a possible source for dissemination of *S. aureus* (Hizeh et al., 1997).

In conclusion, hospital should assess the advantages of routinely culturing personnel, however, in outbreak situation hospital personnel especially young persons may be source of nosocomial infection. *Staphylococcus aureus* colonization varies from one individual to another. Multiple colonizations can occur in healthy individuals outside a hospital environment, without risk factors. *Staphylococcus aureus* in the community is already resistant to various classes of antibiotics. The emergence of multi antibiotic resistant strains in the community was mainly from individuals who had not been hospitalized. This may imply that the emergence of resistance is independent from the influence of antibiotic resistance in the hospitals and antibiotic resistances are no more confined to the hospital. The widespread use of broad-spectrum antibiotics and biocides in the community may also play a part in the selection for antibiotic resistance in *Staphylococcus aureus*. The formulation and implementation of a national drug policy by governments are fundamental to ensure rational drug use. Proper use of drugs has to be promoted by providing objective information and training.

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