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ORAL SUFFERING AND ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* IN HEALTHY CHILDREN IN DENTAL HOSPITAL IN KOLKATA, INDIA

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ABSTRACT

Staphylococcus aureus is a well recognized pathogen associated with a variety of clinical syndrome. The role of Staph aureus in some types of oral disease may be more important than previously recognized. There is increasing evidence that community acquired Staph. aureusinfections are spreading among healthy children. The present study of healthy children was designed to investigate the prevalence of Staphylococcus aureus, MRSA and their rate of resistance to different anti staphylococcal antibiotics. For this study, Gurunanak Institute of Dental Science & Research (Kolkata), selected patients who were suffering from Staphylococcus aureus oral infection. Isolated Staphylococcus aureus was tested for Oxacillin (01 mcg) sensitivity and their antibiotic susceptibility was investigated by using eighteen antibiotics followed by Disk diffusion technique following CLSI method. Out of the 56 specimens collected, 20 (35.7%) were isolated. All the 20 (35.7%) specimens were studied in detail. 5.0 % of the isolates were shown to be methicillin resistant Staph. aureus (MRSA). Percentage (%) of resistance in commonly used oral antibiotics are ampicillin & amoxycillin/clavulanic acid 90%, amoxycillin 70.0%, ofloxacin & ciprofloxacin 50.0%. The MRSA isolates showed multiple drug resistance (MDR), except rifampicin, linezolid and imipenem. In line with more recent surveys, this retrospective study suggests that Staph. aureus may be more frequent isolate from the oral cavity than hitherto suspected. The role of Staph.aureus in several diseases of the oral mucosa merits further investigation.

KEYWORDS

Staphylococcus aureus, Oral infections in children, MRSA, Antibiotic susceptibility.

INTRODUCTION

Staphylococcus aureus is a common human pathogen that causes various skin and mucosal infections. Besides superficial infections, the organism can also cause abscess formation, septicemia, pneumonia, osteomyelitis, and gastroenteritis [1-2].

Although the oral cavity harbors a complex microflora consisting of mostly non-pathogenic microorganisms, it was of interest to investigate the occurrence of *Staph. aureus*. There are several reports of the isolation of this bacterium from the oral region [3-5]. but no detailed characterization was done. Historically antibiotic-resistant strains of *Staph. aureus* were first identified in 1942, just after the begin of clinical treatments with penicillin [6]. In the late fifties, semi-synthetic penicillins, like methicillin, were developed to solve this problem, but only two years later, methicillin resistance was reported [5]. Over the last twenty years, methicillin-resistant *Staph. aureus* (MRSA) strains have emerged as important pathogens, affecting primarily hospitalized patients [7]. This problem seems to be moving beyond the hospital environment. Recent reports showed that the number of community-acquired MRSA, infections had increased [8].

The occurrence of MRSA in the nostril, skin wounds and respiratory tract has been well

documented, but little is known about its presence in the oral cavity or the potential implications for the practice of dentistry[6]. Some reports have showed the persistence of *Staph. aureus* in the oral cavity, especially in children, suggesting that it can serve as a reservoir for MRSA with potential to spread and cause nosocomial infections [4-5]. Organisms referred to as MRSA are actually oxacillin resistant *Staph. aureus* (ORSA). However, as methicillin and oxacillin are similar antibiotics, MRSA is the usually accepted designation.

The present study of healthy children was designed to investigate the prevalence of *Staphylococcus aureus* and MRSA and their rate of resistance to different anti staphylococcal antibiotics.

METHODS:

This was a prospective study conducted for 18 months (March 2011 to August 2012).

STUDY SETTING:

The study was conducted on samples from patients and participants of Gurunanak Institute of Dental Science and Research, Panihati, Kolkata-700114, North 24 parganas, West Bengal, India.

STUDY PARTICIPANTS:

The samples were collected belonged to outdoor patients of Pedodontics department (up to 14 years) of Gurunanak institute of Dental science and Research in Kolkata.

Having explained our goal, doctors were requested to fill information related to oral suffering by *Staph. aureus*. Initial data included name, sex, age and patient complaints. None of the patients, who were related to the case study, were provided with antibiotics (prior to a week).

COLLECTION AND PROCESSING OF SAMPLES:

Oral cavity swabs were collected for case study from oral suffering patients, using sterile oral cavity swabs, (under the guidance of a doctor).

A total of 56 oral cavity swab samples were collected from oral suffering patients. The samples were cultured aerobically in Mannitol salt agar media (Himedia Laboratories Pvt. Ltd.; Mumbai, India). 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, Coagulase test (slide & tube method) and DNase test as described by Cheesbrough[9]. Sensitivity testing using Kirby-Bauer disc diffusion technique [Bauer et al. (1966)] [10]. The following concentration of antibiotic per disc was used as recommended by Clinical Laboratory Standards Institute (CLSI)[11] [Himedia Laboratories Pvt.Ltd.; Mumbai,India]:Amoxycillin(20mcg),Amoxycillin+ Clavulanic acid (20+10 mcg), Ampicillin (10 mcg), Ampicillin+Sulbactam(10+10mcg),Cefpodoxime(10 mcg), Ciprofloxacin (05 mcg), Clindamycin (02 mcg), Erythromycin (15 mcg), Rifampicin (05 mcg), Imipenem (10 mcg), Linezolid (30 mcg), Ofloxacin (05 mcg), Piperacillin (100 mcg), Piperacillin+Tazobactam(100+10mcg), Ticarcillin (75 mcg), Ticacillin+Clavulanic acid (75+10 mcg), Meropenem (10 mcg), Vancomycin (30 mcg), Oxacillin (01 mcg).

Resistance or Susceptibility was reported based on the CLSI guideline. Two hours Tryptone Soya Broth (Himedia Laboratories Pvt.Ltd.; Mumbai, India) (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[9]. Sterile swabs were used to inoculate the test organism onto the sensitivity agar (Mueller Hinton agar media) (Himedia Laboratories Pvt. Ltd.; Mumbai, India). 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 *Staph.aureus* detection (MRSA):

Methicillin-resistance was verified by the CLSI (formerly NCCLS) Oxacillin screening test [12]. 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^{0}C. Isolates were classified as either susceptible Bauer et al. (1966). American Typing Collection (ATCC 25923) of *Staph. aureus* was used as a control strain in antibacterial susceptibility testing.

RESULTS

Table I (a & b): Occurrence of MSSA and MRSA with gender in Pedodontics department (up to 14 years) in Dental hospital.

Table II: Antibiotic disc susceptibility testing was carried out on all the 20 *Staphylococcus aureus* isolates. Strains that exhibited different susceptibility patterns even though isolated from the same patients will be analyzed as separate strains.

A low percentage of the strains were also resistant to oxacillin (5.0%), ampicillin/sulbactam & meropenem (30.0%), clindamycin (40.0%), piperacillin/tazobactam, ciprofloxacin, ofloxacin, vancomycin (50.0%), amoxicillin &ticarcillin/clavulanic acid (70.0%), erythromycin (80.0%), piperacillin, amoxicillin/clavulanic acid, ampicillin &ticarcillin (90.0%) and cefpodoxime (100.0%). All strains were sensitive to imipenem, linezolid & rifampicin. The MRSA isolates showed multiple drug resistance (MDR), except imipenem, linezolid & rifampicin.

[Table I a] MALE PATIENTS:

Total No. of Patients	MSSA	% of MSSA	MRSA	% of MRSA	Total Isolates	% of total Isolates
32	10	31.2	01	3.1	11	34.3

[Table I b] FEMALE PATIENTS:

Total No. of Patients	MSSA	% of MSSA	MRSA	% of MRSA	Total Isolates	% of total Isolates
24	09	37.5	00	0.0	09	37.5

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

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

[Table: II]

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

	Total Isolates : 20			
Antibiotics	S(No.)	R(No.)	%S	%R
Amoxycillin	06	14	30.0	70.0
Amoxycillin/clavulanic acid	02	18	10.0	90.0
Ampicillin	02	18	10.0	90.0
Ampicillin/sulbactam	14	06	70.0	30.0
Cefpodoxime	00	20	0.0	100.0
Ciprofloxacin	10	10	50.0	50.0
Clindamycin	12	08	60.0	40.0
Erythromycin	04	16	20.0	80.0
Meropenem	14	06	70.0	30.0
Imipenem	20	00	100	00
Linezolid	20	00	100	00
Ofloxacin	10	10	50.0	50.0
Oxacillin	19	01	95.0	5.0
Piperacillin	02	18	10.0	90.0
Piperacillin/Tazobactam	10	10	50.0	50.0
Ticarcillin	02	18	10.0	90.0
Ticarcillin/clavulanic acid	06	14	30.0	70.0
Vancomycin	10	10	50.0	50.0
Rifampicin	20	00	100.0	0.0

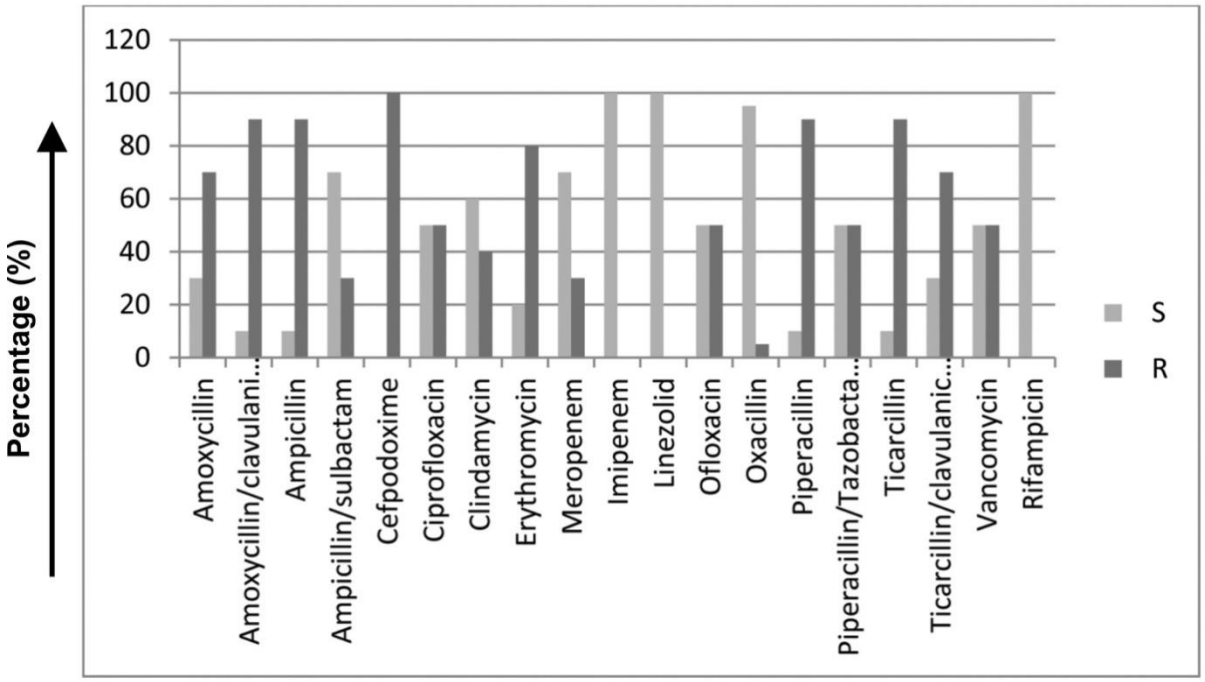

Figure I: Pattern of Staphylococcus aureus susceptibility

***S: Sensitive.**

***R: Resistant.**

Statistical Analysis:

We have performed binary logistic regression to analyze the effect of age and sex on the Methicillin resistant groups and it is turned out to be not significant in both cases (p = 0.28937, p = 0.35339). The data for antibiotic Cefpodoxime, Rifampicin, Imipenem and Linezolid was not considered for the regression analysis since all of the counts were either of the all resistant or all susceptible type. None of the resistance pattern (explanatory variable) of 14 considered test antibiotic has any influence on the resistance pattern of response variable. Again age, sex has no effect on the affection status of *Staphylococcus aureus* Methicillin resistance pattern.

However, Oxacillin is the antibiotic of choice to determine which of the cases are MRSA +ve. That way we have found only one sample from one of the twenty subjects is found to be MRSA positive. Thus we considered the performance as the bench mark and tested which of the antibiotics performed similar or better than Oxacillin. Since some of the expected cell values to be tested were less than 5 we choose to perform one tail Fisher's Exact Test. We observed 3 antibiotics were performing in a similar manner as the Oxacillin do (Statistically not significant p= 0.49999). These are Rifampicin, Imipenem and Linezolid. Rest all 15 test antibiotics are poor performer than Oxacillin with marginal (p=0.047) or very high (p=0.00000000015) statistical significance.

As a matter of fact, for clinical purpose the ideal drug of choice is that which can cure all possible cases independent of its MRSA resistance status. That way we need no statistical test to find the best drug of choice, which are Rifampicin, Imipenem and Linezolid. We already knew that other antibiotics are significantly poor performer than Oxacillin we still performed the one tailed FE test between the Imipenem disc assay data to other two antibiotic's disc assay data. Those two antibiotics are Ampicillin and Meropenem. Again these two antibiotics were found to be significantly poor performer even after Bonferroni correction. The number of resistant cases with Oxacillin is not found to be statistically significant i.e. with this sample set Oxacillin is also similar good performer as those of the best drug of choices. Thus Rifampicin, Imipenem and Linezolid are the drug of choice for the treatment of *Staphylococcus aureus* in case of paediatric cases. Oxacillin can also be a choice. Since it was a benchmark without any doubt we can say Oxacillin is another drug of choice but for all MRSA –ve cases.

DISCUSSION

While the importance of staphylococci as medical pathogens has been recognized for many years, the presence of Staphylococcus species as component of the resident oral flora is controversial but, surprisingly, there have been relatively few detailed studies of the distribution of staphylococci in the mouth [13].

These 18 months long interesting retrospective study reports the isolation of *Staph. aureus* from the orofacial region at a microbiology laboratory in dental hospital, Panihati, Kolkata-700114; West Bengal, India. Demographic and clinical data were collected and the sensitivity of isolates was studied. Out of total 56 study specimens, 20 (35.7%) isolates were found to be *Staph. aureus* positive. 5.0% of the isolates were shown to be methicillin resistant *Staph. aureus* (MRSA). The symptoms most frequently associated with either MSSA or MRSA were erythema, swelling pain, or burning of the mucosa.Common diseases in children's oral cavity are oral mucosititis, angular cheilitis[14] and osteomyelitis of jaw bone[15], caused by *Staphylococcus aureus*. Oral mucosal infection with *Staph.aureus* has recently been incriminated in a severe form of mucositis

reported in some groups with systemic disease such as patients with oral Crohn's disease[16] and geriatric patients[17]. The clinical presentation of staphylococcal mucositis includes colonisation by toxic-producing strains of *Staph. aureus*. In one study, three of five patients with mucositis were colonised by toxic-shock syndrome toxin (TSST)-1-producing strains, suggesting that heavy colonisation of the oral cavity with toxin-producing strains may cause local mucosal damage [17].However, these data indicate the need for further research, particularly in view of the high rate of recovery from patients with mucosal symptoms and the high percentage of oral isolates from previous studies that have been shown to possess virulence factors.

In the present study, in-vitro culture sensitivity pattern of children was assessed for *Staph. aureus* from oral cavity and data from **Table-2 and Figure-1** show that majority of isolated *Staph. aureus* strain from patients 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, rifampicin & linezolid.

Moreover, 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 [18].

The study documents the importance of *Staphylococcus aureus* as important Gram-positive pathogen and increasing resistance in commonly used antibiotics. Although the high cost and inappropriate use of antibiotics have been documented and the long courses of prophylactic antibiotic may lead to increased resistance to antimicrobials, increased incidence of drug reactions and increased dollar costs [19].

Multiple drug resistance of *Staphylococcus aureus* is due to several drug resistant genes in a singleplasmid, each with its own resistance markers. A bacterial cell may carry more than one plasmid With resistance markers. The resistance development in *Staphylococcus aureus* dates back to 1940s. It has a long history of drug resistance can be explainable by the following data [20].

Antibiotic	Year introduced	Reports of resistance
Penicillin	1941	1940s
Streptomycin	1944	1940s
Tetracycline	1948	1950s
Erythromycin	1952	1950s
Methicillin	1959	Late 1960s
Gentamicin	1964	Mid 1970s
Ciprofloxacin	1988	Late 1980s
Vancomycin	1958	1997

Since the development of resistance to antibiotics by the pathogenic strains of *Staphylococcus aureus is* an ever increasing problem, a suitable and possible alternate chemotherapeutic compounds which are of plant origin i.e., phytochemical compounds such as alkaloids, terpenoids, polyphenols and flavonoids may be tried for effective means of controlling drug resistant bacteria like MRSA as has been recently reported [21].

CONCLUSIONS

In line with more recent surveys, this retrospective study suggests that *Staph. aureus* may be a more frequent isolate from the oral cavity than hitherto suspected. A small proportion of the *Staph.aureus* isolates were MRSA. The role of *Staph. aureus* in several diseases of the oral mucosa merits further investigation.

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