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ORAL SUFFERING AND ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS AUREUS IN A 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. The present study has been 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 (1 mcg) sensitivity and their antibiotic susceptibility was investigated by using eighteen antibiotics followed by Disk diffusion technique following CLSI method. Out of the 223 specimens collected, 109 (48.9%) were isolated. All the 109 (48.9%) specimens were studied in detail. 5.5% of the isolates were shown to be methicillin resistant *Staph. aureus* (MRSA). Percentage (%) of resistance in commonly used oral antibiotics are ampicillin 98.1%, amoxycillin/clavulanic acid 73.3%, amoxycillin 45.0%, ofloxacin 48.6% and ciprofloxacin 41.2%. The MRSA isolates showed multiple drug resistance (MDR), except 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, MRSA, Antibiotic susceptibility.**

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INTRODUCTION

Staphylococcus aureus is one of the most commonly found pathogenic bacteria and is hard to eliminate from the human and animals environment.[1] *Staphylococcus aureus* is a Gram positive, non-motile, catalase positive, coagulase positive, facultative anaerobe, involved in causing a number of diseases such as boils, pustules, impetigo, osteomyelitis, mastitis, septicemia, meningitis, pneumonia and toxic shock syndrome.[2,3] Nosocomial infections of which *Staph. aureus* is a typical example, are known to account for morbidity and mortality of millions of patients annually worldwide.[4] *Staph. aureus* is considered 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 temperatures (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. [3] *Staph. aureus* is known to be notorious in their acquisition of resistance to new drugs and continues to defy attempts at medical control. [3] Many strains of *Staph. aureus* carry a wide variety of multi-drug resistant genes on plasmids. The resistance of *Staph aureus* isolates from different parts of the world to commonly used antibiotics has been widely reported.[5,6] *Staphylococcus aureus* is a well recognized pathogen associated with a variety of clinical syndromes. With the exception of angular cheilitis,[7] some endodontic infections [8,9,10,] such as osteomyelitis of the jaw[11] and parotitis[12,13] and a form of oral mucositis in elderly were noticed in patients. The bacterium is commonly regarded as a transient coloniser of the oral cavity and often disregarded when isolated from clinical specimens. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a challenge for all health care institutions. Previously limited to large institutions, outbreaks of MRSA are now quite common in all hospital settings. [15, 16] These strains are not only resistant to multiple antibiotics, but also act as a reservoir for multiple drug-resistant genes. The present study 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 15 months (March 2011 to May 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 collected belonged to outdoor patients of various departments 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, patient complaints, past medical history, oral habit (smoking or others), and use of mouthwash etc. All the above information was collected by a questionnaire. Past medical history of underlying diseases that might increase the chance of colonization, such as, chronic renal disease, independent diabetes mellitus and dialysis were detected.[17] 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 223 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 (used of Mannitol salt agar), Gram staining, Catalase, Coagulase test (slide & tube method) and DNase test as described by Cheesbrough.[2] Sensitivity testing using Kirby-Bauer disc diffusion technique [Bauer et al. (1966)].[18] The following concentration of antibiotic per disc was used as recommended by Clinical Laboratory Standards Institute (CLSI).[19] [Himedia Laboratories Pvt.Ltd.; Mumbai, India]: Amoxycillin(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 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.[2] 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.[20] 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 35^0C. 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 1
Occurrence of MRSA and MSSA in different departments in Dental hospital

Department	TotalNo.of Sample	MSSA	MRSA	Total Isolates
OPD	76	41	00	41
Oral pathology & Microbiology	43	14	03	17
Pedodontics	43	14	01	15
Oral Surgery& Maxillofacial	61	34	02	36
Total	223	103	06	109
Percentage (%)		46.2%	2.7%	48.9%

MSSA: Methicillin-sensitive Staph. aureus.
MRSA: Methicillin-resistant Staph. aureus.
OPD: Outpatient department.

Table 2
Occurrence of MSSA and MRSA with gender in different departments in Dental hospital

Department	Male Patients				Female Patients			
	TOTAL	MSSA	MRSA	Total isolates	TOTAL	MSSA	MRSA	Total isolates
OPD	41	21	00	21	35	20	00	20
Oral Pathology & Microbiology	22	09	01	10	21	05	02	07
Pedodontics (0-14 years)	22	07	01	08	21	07	00	07
Oral Surgery& Maxillofacial	37	22	01	23	24	12	01	13
Total	122	59	03	62	101	44	03	47
Percentage(%)		48.4%	2.4%	50.8%		43.5%	3.0%	46.5%

Table 3
Occurrence of MSSA and MRSA with age group and gender

Age group(Year)	Male patients				Female patients			
	Total No. of Patients	MRSA	MSSA	Total Isolated	Total No. of Patients	MRSA	MSSA	Total Isolated
0-10	14		05	05	13		05	05
11-20	12	01	05	06	11		05	05
21-30	13		06	06	12		06	06
31-40	11		06	06	17		08	08
41-50	34	02	21	23	19	02	06	08
51-60	13		06	06	13		06	06
61 and above	25		10	10	16	01	08	09
Total	122	03	59	62	101	03	44	47
Percentage (%)		2.4%	48.4%	50.8%		3.0%	43.5%	46.5%

Antibiotic susceptibility patterns

Antibiotic disc susceptibility testing was carried out on all the 109 *Staphylococcus aureus* isolates. Strains that exhibited different susceptibility patterns even though isolated from the same patients will be analyzed as separate strains (Table 4).

A low percentage of the strains were also resistant to linezolid (2.8%), oxacillin & rifampicin (5.5%), ampicillin/sulbactam (17.4%), meropenem (27.5%), piperacillin/tazobactam (30.3%), clindamycin (31.1%), ciprofloxacin(41.2%), vancomycin (44.0%), amoxycillin(45.0%), ofloxacin (48.6%), ticarcillin/clavulanic acid (52.3%), piperacillin (58.7%%), amoxycillin/clavulanic acid (73.3%), erythromycin (74.3%), ticarcillin(77.0%), cefpodoxime (97.2%) and ampicillin (98.1%). All strains were sensitive to imipenem. The MRSA isolates showed multiple drug resistance (MDR), except imipenem & linezolid.

Statistical Analysis

Binary logistic regression were performed to analyze the effect of age and sex and tobacco habits on the methicillin resistant groups and it is turned out to be not significant in each cases (p_a=0.219854, p_s = 0.597972 and 0.311357). The data for antibiotic imipenem was not considered for the regression analysis, as all of the counts were zero. None of the resistance pattern (explanatory variable) of 18 test antibiotic has any influence on the resistance pattern of response variable (methicillin resistance). Again age, sex or tobacco habit has no effect on the status of *Staphylococcus aureus* methicillin resistance pattern. The only exception is ampicillin/sulbactam(after multiple corrections).

Fisher's exact test was performed to find out the drug of choice pair. (Since some of the cell values to be tested were less than 5). As a matter of fact, for the studied data set it needs no statistical test to find the best drug of choice, which is imipenem (since none of the study bacterial culture from different patients are resistant). To know the other drugs of choice and to control the multiple testing related issues, we have sorted the resistant samples data in a smallest to largest manner and then performed the Fisher's exact test between the imipenem disc assay data to other sorted data as per rank. R package was used for this analysis. Bonferroni correction was performed, and later checked if the p value is still significant. (If not, then try the next test). Once it is significant that, even after Bonferroni correction, for more confidence we performed another test with the next rank data. Since it was already sorted, rest of the tests would automatically be significant (Holm's Correction).[21] In this way we found that oxacillin and ampicillin/ sulbactam was found to show significantly greater number of resistance cases in this study bacterial samples in comparison to imipenem. Again number of resistant cases with linezolid and rifampicin is not found to be statistically significant. Thus along with imipenem, linezolid and rifampicin also can be drug of choice for the treatment of *Staphylococcus aureus.*

Test Antibiotic	expected P value after Bonferroni correction	Observed P value
Imipenem		
Linezolid	0.05	0.1233
Rifampicin	0.025	0.0298
Oxacillin	0.016666667	0.0146
Ampicillin/Sulbactam	0.0125	0.0001
Meropenem	0.01	

To be confident, we performed another level of one tailed Fisher's exact test. Since Oxacillin is the antibiotic which is used to define MRSA strains of *Staphylococcus aureus* the drug of choice must have to be significantly better in reducing the frequency of antibiotic resistance cases. So we performed the test using same sort-rank strategy as already explained. Once again, we found that imipenem and linezolid were significantly more efficient in reducing the number of resistant cases in the study samples, where as rifampicin is not found to be significant in this case.

Test Antibiotic	expected P value after Bonferroni correction	Observed P value
Imipenem	0.016666667	0.0146
Linezolid	0.025	0.2493
Rifampicin	0.05	0.5
Oxacillin		
Ampicillin/Sulbactam	0.05	0.0321
Meropenem	0.025	0.0001

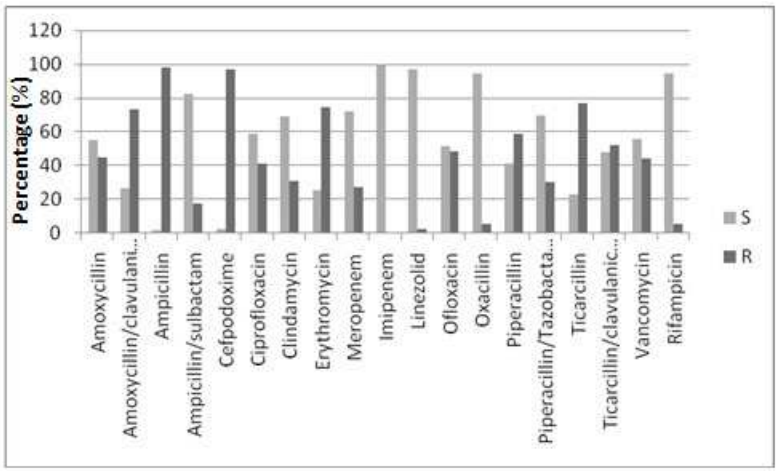
However, ampicillin/sulbactam and meropenem (best in the rest lot) are found to show significantly more number of resistance cases even in comparison to oxacillin. Automatically, this implies that the performance of other antibiotics would be even worse.

P.S: During analysis we categorize the age into six groups, viz. 0-14,15-30, 30-45, 45-60, 60-75 and above 75; Whereas the tobacco have been categorized into four (45-60 years) viz. no habit, smoker, chewer and smoke-chewers. All the resistant counts were coded as 1 and the susceptible counts were coded as 0.

Table 4

Percentage susceptibility of isolated Staphylococcus aureus to tested antibiotic:

	Total Isolates : 109			
Antibiotics	**S(No.)**	**R(No.)**	**%S**	**%R**
Amoxycillin	60	49	55.0	45.0
Amoxycillin/clavulanic acid	29	80	26.7	73.3
Ampicillin	02	107	1.9	98.1
Ampicillin/sulbactam	90	19	82.6	17.4
Cefpodoxime	03	106	2.8	97.2
Ciprofloxacin	64	45	58.8	41.2
Clindamycin	75	34	68.9	31.1
Erythromycin	28	81	25.7	74.3
Meropenem	79	30	72.5	27.5
Imipenem	109	00	100	00
Linezolid	106	03	97.2	2.8
Ofloxacin	56	53	51.4	48.6
Oxacillin	**103**	**06**	**94.5**	**5.5**
Piperacillin	45	64	41.3	58.7
Piperacillin/Tazobactam	76	33	69.7	30.3
Ticarcillin	25	84	23.0	77.0
Ticarcillin/clavulanic acid	52	57	47.7	52.3
Vancomycin	61	48	56.0	44.0
Rifampicin	103	06	94.5	5.5



***S: Sensitive.**
***R: Resistant.**

Figure 1
Pattern of Staphylococcus aureus susceptibility

DISCUSSION

Staphylococcus aureus is recognized as an important bacterial pathogen contributing towards hospital infection, globally. *Staphylococcus aureus* causes localized infection spreading into the blood stream. Despite the use of potent antibiotic still high mortality exist in case of *Staphylococcus aureus* infection.

These 15 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 223 study specimens, 109(48.9%) isolates were found to be *Staph. aureus* positive. 5.5% 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. 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 [22] and geriatric patients.[14] 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.[14] 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 was assessed for *Staph. aureus* from oral cavity and data from Table-4 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 & linezolid. In this study rifampicin is sensitive to 94.5%. This high rate of sensitivity may be because rifampicin is not in common use and is normally used in the treatment of tuberculosis caused by *Mycobacterium tuberculosis.*

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. [23]

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. [24]

Multiple drug resistance of *Staphylococcus aureus* is due to several drug resistant genes in a single plasmid, 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. [25]

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. [26]

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* isolate was MRSA. The role of *Staph.aureus* in several diseases of the oral mucosa merits further investigation.

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