

Research Article**Methicillin Resistant *Staphylococcus aureus* and Antibiogram in Physical and Mentally Handicapped Children of Anoopam Mission, Anand, Gujarat****Navneet Kumar Singh¹, Kiran Kalia², Jagdish S. Patel³**¹Shree P.M Patel College of Paramedical Science & Technology, Anand²Department of Bioscience, Sardar Patel University³Dept. of Biochemistry, CHARUST University**ABSTRACT**

To determine the prevalence of and the risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in Physical and mentally handicapped children of Anoopam Mission, Anand. 115 physically and mentally Handicapped childrens studying at Anoopam Mission Anand. After informed consent was obtained, all participants had their nares swabbed, some personal data collected, or both. All swabs were examined for growth of MRSA. All *S. aureus* isolates underwent oxacillin susceptibility testing and polymerase chain reaction for demonstration of the *mecA* gene. Swabs from 115 children show 13 *Staphylococcus aureus* and 1 MRSA strains, contributing to a prevalence rate of 0.07%. Significant risk factors for MRSA carriage in the multivariate analysis were the admission to a hospital during the preceding 3 months, or stay in a medium-size nursing home. One predominant MRSA strain could be detected in 1 of the 13 *Staphylococcus aureus* carriers. The prevalence of MRSA in Physical and Mentally handicapped children of Anoopam Mission, Anand. is still very low. These residents seemed to acquire their MRSA in the hospital and transfer it to their nursing home. Apart from well-known risk factors for the acquisition of MRSA, This might be due to an increased use of antimicrobials in nursing homes of a certain size.

INTRODUCTION

Staphylococcal infections are very common in the pediatric age group, accounting for most superficial and deep-seated soft tissue infections. Traditionally community-acquired strains are sensitive to methicillin, and usual antibiotic regimens for these infections include beta-lactams with appropriate anti-staphylococcal coverage. In the 1990s, methicillin-resistant *Staphylococcus aureus* strains were increasingly recognized in the community, and varying rates of community-acquired MRSA infection and nasal colonization in both adults and children have been reported. Data are available on MRSA colonization rates in both pediatric hospitals and child day-care centers. [1] The one having physical or mental disability, more often than not, becomes a parasite on the family and the community making life a miserable, experience. Mental retardation refers to sub average general intellectual functioning which originates during the development period and is associated with impairment in adaptive behaviour. The mentally retarded from childhood experiences unusual difficulties in learning which affects his capacities for adjustment in day to day. *Staphylococcus aureus* appears to become drug resistant more readily than the most other bacteria. Penicillin was the first antibiotic used for staphylococcal infection and penicillin resistance appeared shortly after its introduction. This was followed by the resistance to co-trimoxazole, ampicillin, amoxicillin and tetracycline. Resistance to erythromycin and chloramphenicol also occurs but to a lesser extent than other antibiotics. Strains resistant to more than one antibiotic are now by rule. [3] Methicillin resistant *Staphylococcus aureus* is an increasing problem in health care facilities. [4] Infection with this MRSA strains, which are resistant to wide range of antibiotics, is associated with considerable morbidity and mortality. [5] The spread of MRSA may indicate that recommended preventive strategies in these countries are either inadequate or improperly implemented. [6] *Staphylococcus aureus* nasal carriage, present in about 20% of the general population, has been identified as a risk factor for the subsequent development of community acquired and nosocomial staphylococcal infections. [7] Transmission of MRSA occurs primarily from colonized or infected patients to other patients or staff, or vice-versa.[8-10]. Widespread antibiotic use has resulted in increased frequency of clinically important bacteria acquiring single or multiple antibiotic resistance. [11] The rationale for our study was to assess the prevalence of MRSA carriage and to investigate possible risk factors for

MRSA colonization in physically and mentally retarded childrens at Anoopam Mission, Moigri Anand.

MATERIALS AND METHODOLOGY

Study design

Total 115 nasal swabs were obtained from physically and mentally retarded children at Anoopam Mission, Anand. Sterile dry cotton swab were used for the collection nasal swab. For collection of nasal swab, the swabs were rubbed by rotating 5-7 times over the inner wall of ala and nasal septum and immediately processed for culture and isolation.

Culture of clinical specimen and nasal swab

The specimens were cultured on the Phenol Red Mannitol Salt Agar (a selective medium for *Staphylococcus aureus*) by streaking & the specimen collected in swabs were processed within one hour after collection as per the conventional techniques. The culture plate incubated at 37°C for 24-48 hours in incubator.

Isolation and Identification of *Staphylococcus aureus*:- Mannitol fermenting yellow colored colony is selected and subject to Gram stain and subcultured on the 5% Blood Agar to observe the β -hemolysis. The isolates showing gram-positive cocci in clusters and β -hemolysis were subjected to catalase and coagulase test by slide and test tube technique using undiluted and 1:6 diluted human plasma respectively.

Screen test for MRSA

A suspension equivalent to 0.5 Mac Farland was prepared from each strain. A swab was dipped and streaked over an area of approximately 2x2.5 Cm. on the surface of a Mueller-Hinton agar supplemented with 4% NaCl and 6 mcg/ml Methicillin (Sigma-Aldrich). Plates were incubated overnight at 37 °C. A growth indicates that the strain is methicillin resistant.

Sensitivity to other antibiotics:-

AST (Antibiotic susceptibility testing) pattern were studied by Kirby Bauer Disc diffusion techniques as per CLSI (Clinical Laboratory Standards) Guidelines formerly National Committee for Clinical Laboratory Standards. The inoculum of the isolates equivalent to 0.5 Mc Farlands unit were swabbed onto the Muller-Hinton Agar Plate and then the antibiotic disc were placed on it and incubated overnight at 37°C. The zone of inhibition is interpreted acc. to CLSI Guidelines. The antibiotics used for testing were **Dodeca Staphylococci-1(HIMEDIA DE048):** PenicillinG(10units), Azithromycin(15mcg),Erythromycin(15mcg),Clarithromycin(15mcg),Linezolid(30mcg),CoTrimoxazole(25mcg),Vancomycin(30mcg),Ciprofloxacin(5mcg),Gatifloxacin(5mcg),Ofloxacin(5mcg),Clindamycin(2mcg). **Dodeca Staphylococci-2(HIMEDIA DE049)** Moxifloxacin(5mcg),Gentamicin(10mcg),Rifampicin(5mcg),Lomefloxacin(10mcg),Norfloxacin(10mcg),Teicoplanin(30mcg),Nitrofurantoin(300mcg),Ampicillin/Sulbactam(10/10mcg),Piperacillin/Tazobactam(100/10mcg).

RESULT

Screening and Identification of *Staphylococcus aureus* strain

Total 115 Nasal swab of physically and mentally retarded children were studied, obtained from Anoopam Mission, Mogri, Anand from July'2010 to November'2010. Out of which 13 were identified as staphylococcus aureus. The specimens were obtained from Anoopam mission, Anand.]

Table 1:- Total No. of specimens

Total clinical samples obtained	No. of <i>Staph. aureus</i> isolates
115	13

Table 2: Total No. of isolates of *Staphylococcus aureus* and CONS

	(n=13)
Staphylococcal species	20 (17.4%)
<i>Staphylococcus aureus</i>	13 (11.3%)
MRSA	00 (00%)
Coagulase negative Staphylococci (CONS)	07 (6.08%)

Culture on Mannitol Salt Agar

It is the selective media for *Staphylococcus aureus*. *S. aureus* ferments mannitol. All the thirteen isolates were streaked on the MSA plates. All were found to be mannitol fermenter. The colonies were yellowish in color surrounded by yellow colored zone



Fig 1: Plate showing Mannitol fermentating colony of *S. aureus*

Fig 2: Plate showing Mannitol non-fermenting pink colony of CONS

For the conformation, All thirteen selected isolates were subjected to Catalase, Coagulase test and streaked onto the Blood Agar. All isolates showed catalase and coagulase test positive. Also they showed β -hemolysis on Blood Agar (figure 2 & 3). All the 13 samples of *Staphylococcus aureus* strains were tested in vitro to determine their antibiotic susceptibility pattern by Kirby Bauer antibiotic disc diffusion method.

Majority of the strains showed multiple drug resistance to the drug tested. Study of Multi-drug resistance among 13 *S. aureus* isolates showed the level of multi-drug resistance, it is shown in Table No 4 About 23.07% of the isolates were resistant to at least 11 antibiotics, 15.38% were resistant to at least 1,5 and 4 antibiotic and 7.69% were resistant to at least 3,6,9 and 10 antibiotics.

Table 3:- Distribution of antibiotic resistance among different antibiotics of *Staphylococcus aureus* strain isolated from nasal swab of Physically and Mentally retarded children.

Antibiotic	Resistant		Susceptible	
	N	Percentage(%)	N	Percentage(%)
P	13	100.00%	0	0.00%
AZM	2	15.38%	11	84.62%
E	3	23.07%	10	76.93%
CLR	3	23.07%	10	76.93%
LZ	3	23.07%	10	76.93%
COT	7	53.84%	6	46.16%
VA	0	00%	13	100%
CIP	4	30.77%	9	69.23%
GAT	4	30.77%	9	69.23%
OF	3	23.07%	10	76.93%
CD	1	7.69%	12	92.31%
MO	5	38.46%	8	61.54%
GEN	1	7.69%	12	92.31%
RIF	0	0.00%	13	100.00%
LOM	7	53.84%	6	46.16%

NX	7	53.84%	6	46.16%
TEI	1	7.69%	12	92.31%
NIT	6	46.16%	7	53.84%
A/S	0	0.00%	13	100.00%
P/T	2	15.38%	11	84.62%

DISCUSSION

The emergence of MRSA in the community is of great importance and is the subject of multiple studies in a variety of clinical settings and from many parts of the world. This study was designed to answer several specific questions. First, the actual rate of *Staphylococcus aureus* colonization, whether sensitive or resistant to methicillin, Physically and Mentally hretarded children was as yet unknown. Our study was conducted at Anoopam Mission, Mogri Anand. Methicillin-resistant *S. aureus* (MRSA) has remained a major cause of nosocomial disease world-wide causing 50% or more of hospital-acquired *S. aureus* infections in several countries (Aires de Saisa and de Lencastre, 2004; Tietz et al., 2005; Wolter et al., 2008). The emergence of community-acquired MRSA that is capable of causing infections in otherwise healthy people has been reported (Gorak et al., 1999; Coronado et al., 2007; Diep et al., 2008). These figures of low MRSA colonization are in the same range as those reported in other studies, such as those of Nakamura et al. [10] recently reported from Nashville, but are far lower than those reported by others [12-14]. Nursing homes and long-term care facilities are usually considered as reservoirs for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers. Actually, there are major differences in MRSA carriage between institutions, with variations from 1% to more than 30%. Overall there is a low incidence of MRSA infection in these institutions, even though carriage is associated with a higher risk of subsequent MRSA infection, with high mortality rates. The anterior nares are considered to be primary colonization site and approximately 30% of healthy people carry the bacteria in the anterior nares. [15] Nasal swabs were found to be colonized with at least one staphylococcal species in 92.8% of the cases reported by Karsten Becker. [16] His results shows overall, 52 *S. aureus* isolates encompassing 47 MSSA and 5 (9.61%) MRSA isolates. In the study done by Yechiel Schlesinger et al. 23.6 % colonization rate, 2.6% of which were MRSA which is less in our study showing 17.4%. *S. aureus* and 0% MRSA. The highest level of resistance of *S. aureus* strain has been observed with Penicillin-G (100%), which is in accordance with the reports of Tiwari et al. [14] Azithromycin (15.38%), Erythromycin (23.07%), Clarithromycin (23.07%), Linezolid (23.07%), CoTrimoxazole (53.84%), Ciprofloxacin (30.76%), Gatifloxacin (30.76%), Ofloxacin (23.07%), Clindamycin (7.69%), Moxifloxacin (38.46%), Gentamicin (7.69%), Rifampicin (0%), Lomefloxacin (53.84%), Norfloxacin (53.84%), Teicoplanin (7.69%), Nitrofurantoin (46.15%), Ampicillin/Sulbactam (0%), Piperacillin/Tazobactam (15.38%). There is not too much reference data for finding prevalence of MRSA in physically and mentally retarded children. Our result shows that the prevalence rate of MRSA is about 0.07%. So, the present study shows comparatively nil prevalence rate of MRSA in Anand District. Data for physically and mentally retarded children is not sufficient to study and co-relate our study. This is a new work first time studied in Anand district of Gujarat.

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