ORIGINAL ARTICLE

NASAL CARRIAGE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS AMONG INPATIENTS OF JIMMA HOSPITAL, SOUTH WESTERN ETHIOPIA

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ABSTRACT

BACKGROUND*: Staphylococcus aureus is one of the major causes of community and hospital acquired infections. The emergence of methicillin resistant strains of Staphylococcus aureus in the hospitals and the community is a serious health problem. The aim of this study was to determine the nasal carriage and antimicrobial resistance patterns of Staphylococcus aureus isolates in Jimma Hospital inpatients.*

METHOD: This *cross sectional study was conducted from January 22 to February 18, 2002. Nasal swabs from 152 inpatients were taken using simple systematic sampling method. Specimens were cultured on mannitol-salt and blood agars. Antimicrobial tested included ampicillin, Oxacillin, Erythromycin, trimethoprim-sulphamethoxazole, chloramphenicol, gentamycin, kanamycin, and clindamycin. Susceptibility tests were carried out on Muller Hinton agar using modified Kirby-Bauer agar diffusion method. Questionnaire was employed to collect data on risk factors for nasal colonization of methicillin resistant Staphylococcus aureus.*

RESULTS: *Out of the total 152 inpatients enrolled in the study, 79(52%) were males and 73(48%) females with a sex ratio of 1:08. One hundred forty-four swabs cultured on mannitol salt agar grew Staphylococcus species out of which 85(59%) were coagulase positive and 59 (41%) were coagulase negative. Antibiotic sensitivities of 85 Staphylococcus aureus isolates to ampicillin, chloramphenicol, trimethoprim-sulphamethoxazole, oxacillin, erythromycin, kanamycin, gentamycin and clidamycin showed resistance pattern of 87.1%, 70.6%, 68.2%, 51.8%, 42.4%, 16.5, 15.3% and 12.9% respectively. 51.8% of the isolates were methicillin resistant Staphylococcus aureus and 72.9% were multi resistant. There was an association between known underlying conditions, previous hospitalization and previous antibiotic use with carriage state of Staphylococcus aureus; P< 0.05.*

CONCLUSION: *This study showed a high rate of nasal carriage of methicillin resistant Staphylococcus aureus among inpatients. Most methicillin resistant Staphylococcus aureus strains are resistant to other beta-lactam antibiotics. Hence, antimicrobial susceptibility tests should be conducted before prescribing antibiotics in order reduce the transmission route and control the problem.* **[Ethiop J Health Sci 2003; 13(2): 107-116].**

Key words: Methicillin, Staphylococcus aureus, Nasal carriage, antibiotic resistance.

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INTRODUCTION

Staphylococcus aureus, a gram-positive commensal of the nose and skin, is carried by one third (30-50%) of the population. The organism causes different infections ranging from mild skin infections and food poisoning to life threatening pneumonia, endocarditis, sepsis and osteomyelitis (1).

S. aureus overcomes antibiotic effectiveness (2, 3). Strains of *S. aureus* that were fully sensitive to penicillin and other antibiotics became penicillinase-producers, which dominated the entire *S. aureus* population in the hospital and the community. This renders penicillin and ampicillin useless as antibiotics for these organisms. Penicillinase stable β-lactams (represented by methicillin, oxacillin and flucloxacillin) developed as a first line anti-staphylococcal drugs, soon emerged methicillin resistant *S aureus* (MRSA) invalidating almost all antibiotics including the most potent β-lactams (4). Waves of epidemic of MRSA strains since 1970 have spread widely, becoming the main cause of nosocomial infections worldwide.

Methicillin inhibits the penicillin binding proteins (PBPs) including the critical PBP2, which are all involved in the synthesis of the cell wall (5). In the presence of methicillin, MRSA strains began to express their methicillin-tolerant PBP'2, which compensates for the inhibited enzyme. PBP'2 is encoded by the mecA gene carried by a highly mobile genetic element SCCmec (Staphylococcal Casset Chromosome mec), which is disseminated among strains with apparent ease (6).

The prevalence of MRSA differs between countries. In New York City, MRSA accounts for 30.0% of nosocomial infections and 50.0% of associated deaths (7). The proportion of MRSA among staphylococci spp isolated in hospitalized patients ranged from <1% in Scandinavia to >30% in Spain, France and Italy (8). The first report of MRSA from Ethiopia was made from 1987 to 1988 from clinical specimens and the overall MRSA isolation rate was 31% while 71% were β-lactamase producers (9). In addition, it was found that 80.0% of the MRSA strains were multiple-drug resistant. In a retrospective study conducted at Jimma regional laboratory from 1990-1991, the most common isolates among the common pathogens was *S aureus*, which is resistant to common antimicrobials (10). In another study, 40.0% of the *S. aureus* isolates were MRSA, which were also resistant to vancomycin (25.0%), chloramphenicol (38.0%), erythromycin (50.0%), clindamycin (50.0%) and penicillin (50.0%) (11). A study conducted on nasal carriage of surgical staffs and environmental contamination of Black Lion hospital revealed that the isolation rate of MRSA was 27.6% (12).

Many studies conducted in different parts of Ethiopia on antimicrobial sensitivity patterns of common pathogens have so far indicated the presence of multiple-antimicrobial resistance from different clinical samples with the common isolate being *S aureus*.

The current study was aimed to determine the nasal carriage of MRSA by inpatients

MATERIALS AND METHODS

A cross-sectional study was conducted in Jimma Hospital inpatients. After verbal consent was obtained from patients, nasal swabs were collected from the anterior nares of each patient during January 22 to February 18, 2002. One hundred fifty two inpatients from maternity, gynecology, surgery and medical wards were enrolled using simple systematic sampling technique from inpatients registration card. Every other patient was included after

isolating the first patient by lottery method. The study subjects were also provided with questionnaire regarding the risk factors for MRSA acquisition such as previous hospitalization, antibiotic use within past year and history of chronic illness or underlying conditions (heart disease, cystic fibrosis, dialysis or other long term medical problems and medications) and history of hospital stay.

Laboratory methods

Nasal swabs and culture: Sterile cotton swabs (culture swab transport system; Difco; Detroit) were used to collect swabs from the anterior nare of the subjects selected. Specimens for culture were obtained by firmly rotating a new pre-moistened cotton-tipped swab in each anterior nare. Swabs were inoculated directly on to MSA (Oxoid) within four hours of sampling. Plates were then incubated at 37^0c for 24 hours to 48 hours and left at room temperature to stimulate pigment formation. Individual colonies were streaked on to sheep blood agar (5% trypticase soya agar; oxoid) and incubated at 37^0c overnight. Morphologically distinct colonies were tested for the production of coagulase using slide method. Mannitol positive and coagulase positive staphylococci were taken as *S aureus* and mannitol negative and coagulase negative colonies as other staphylococci.

***Susceptibility testing*:** was carried out by using modified Kirby-Bauer agar diffusion method with antibiotic discs ampicillin (10μg), Oxacillin (1μg), Erythromycin (15μg), trimethoprim-sulphamethoxazole (25μg), chloramphenicol (30μg), gentamycin (10μg), kanamycin (30μg), and clindamycin (2μg) that were of the same brand on Mueller-Hinton agar (13). Control stains of *S. aureus* ATCC 25923 were used for quality control.

Data were cleaned, edited and entered in to computer and analyzed using SPSS for windows version 11.0. Statistical tests for significance (two sample t-test and X^2-test were employed where applicable at the level of significance 5%.

RESULTS

A total of 152 study subjects were enrolled in this study; 79(52.0%) were males and 73(48.0%) were females with a sex ratio of 1:08. The ages of study subjects ranged from 45 days to 78 years in five of the wards. The median duration of hospitalization for MRSA carriers was 21.8 days while it was 5.3 days for MSSA carriers and 1.2 for non carriers. Of the 152 inpatients from whom nasal swabs were collected and cultured on MSA, 144 (94.7%) showed growth of Staphylococci out of which 85 (59.0%) were *S aureus* and 59(41.0%) were found to be other species of Staphylococci while 8(5.3%) were non-carries. The carriage state among different wards is summarized in table 1.

Table 1. Isolation rate of *S. aureus* and other *Staphylococcus species* among 152 nasal swab cultures from Respective Wards in Jimma Hospital, January February 2002.

Ward	Number of nasal swab cultures tested	S. aureus isolated	Other species of staphylococcus	No of total positive cultures
		No (%)	No (%)	No (%)
Maternity	12	3(27.3)	8(72.7)	11(91.7)
Gynecology	16	9(56.3)	7(43.7)	16(100)
Pediatrics	27	14(53.9)	12(46.1)	26(96.3)
Surgery	57	36(67.9)	17(32.1)	53(93.0)
Medicine	40	23(60.5)	15(39.5)	38(95.0)
Total	152	85(59.0)	59(41.0)	144(94.7)

The Kirby-Bauer sensitivity test for the 85 isolates of S aureus against eight commonly used antibiotics showed resistance rate of 87.1% for ampicillin, 70.6% for chloramphenicol, 68.2% for trimethoprim-sulphamethoxazole, 51.8% for oxacillin, 42.4% for erythromycin, 16.5% for kanamycin, 15.3% for gentamicin and 12.9% for clindamycin (Table2).

Table 2. Antibiotic sensitivities of 85 *S. aureus* isolates against commonly used antibiotics form nasal swab cultures in Jimma Hospital, January 22 -February 18,2002.

Type of antibiotic tested	Staphylococcus aureus			
	Resistant		Sensitive	
	No	%	No	%
Oxacillin	44	51.8	41	48.2
Ampicillin	74	87.1	11	12.9
Chloramphenicol	60	70.6	25	29.4
Trimethoprim Sulphamethoxazole	58	68.2	27	31.8
Erythromyicin	36	42.4	49	57.6
Kanamycin	14	16.5	71	83.5
Gentamycin	13	15.3	72	84.7
Clindamycin	11	12.9	74	87.1

Resistance to oxacillin was separately analyzed among the isolates of *S. aureus* and summarized in table 3. It was found that 44(51.8%) were MRSA and 37 (43.5%) were MSSA out of the total isolates. Surgical ward had the highest isolates of MRSA 23 (63.9%) followed by medical ward 13(56.5%), pediatrics ward 6(42.9%), and gynecology ward 2(22.2%). No MRSA strain was isolated from the maternity ward.

Table 3. The rate of isolation of methicillin resistant and sensitive strains of *Staphylococcus* from inpatients in different wards of Jimma Hospital, January-February 2002

Ward	No of Nasal swab cultures	S aureus	MRSA	MSSA	No of total positive culture
		No (%)	No (%)	No (%)	No (%)
Maternity	12	3(27.3)	-	3(100)	11(91.7)
Gynecology	16	9(56.3)	2(22.7)	7(77.8)	16(100)
Pediatrics	27	14(53.9)	6(42.8)	8(57.2)	26(96.3)
Surgery	57	36(67.9)	23(63.9)	13(36.1)	53(93.0)
Medicine	40	23(60.5)	13(56.5)	10(43.5)	38(95.0)
Total	152	85(59.0)	44(52)	41(48.0)	144(94.7)

MRSA= Methicillin Resistant *S. aureus*; MSSA= Methicillin Sensitive *S. aureus*

The resistance pattern of 85 *S. aureus* showed that most of the isolates were resistant to at least one antimicrobial agent and 68(80.0%) of the isolates were multi-resistant (resistant to three or more antimicrobial agents) [Table 4].

Comparison of Variables of *S. aureus* carriers with non- carriers showed significant association with previous hospitalization (P=0.01), previous antibiotic use (P=0.04), and underlying conditions (p=0.025). It was found that sex has no significant association with *S. aureus* colonization (p=0.44) [table 5].

Table 4. Antibiograms of *Staphylococcus aureus* isolates of different wards of Jimma Hospital, January-February 2002

Antibiotics	No and % Resistant Strains	
	No	%
Amp	8	9.4
C	1	1.2
Amp,Ox	5	5.9
Amp, C	3	3.5
Sxt, C	3	3.5
Amp, Sxt,C	4	4.7
Amp, Sxt, Ox	3	3.5
Amp, C, Ox	1	1.2
C, Sxt, CN	2	2.4
C, E, Ox, Sxt, Amp	8	9.4
C,E, Ox, Sxt, CN	6	7.1
C,E, Ox, Amp, K	5	5.9
C,E, Sxt, CN, DA	3	3.5
C,E, Sxt, Amp, DA	4	4.7
C, Ox, Sxt, Amp, DA	2	2.4
Ox, E, C, Sxt, Amp, DA	2	2.4
Ox, E, C, Sxt, Amp, CN	2	2.4
Ox, E, C, K, DA, CN	1	1.2
TOTAL	85	100

*Amp = Ampicillin, Ox = Oxacillin C = Chloramphenicol, Sxt = trimethoprim-sulphamethoxazol, CN= gentamicin, DA= clindamycin, E=erythromycin, K= Kanamycin.

Table 5. Nasal carriage among inpatients with various predisposing factors at Jimma Hospital, January- February 2002

Variable	S aureus (n=85) No (%)	No S aureus (n=67) No (%)	X^2 P- value	MRSA (n=44) No (%)	MSSA (n=37) No (%)	X^2 P- value
Male	45(53)	34(51)	x^2 =0.01	21(48)	22(58.5)	X^2=0.01
Female	40(47)	33(49)	p=0.92	23(52)	15(41.5)	P=0.44
Previous Hospitalization						
Yes	65(77)	22(33)	X^2 =27.39	39(89)	24(63.4)	X^2=6.17
No	20(23)	45(67)	P=0.000	5(11)	13(36.6)	P=0.01
Previous antibiotic use						
Yes	69(81)	19(28)	X^2 =40.74	40(91)	27(70.7)	X^2=4.49
No	16(19)	48(72)	P=0.000	4(9)	14(29.3)	P=0.04
Known underlying condition						
Yes	68(80)	24(36)	X^2=28.79	39(89)	24(70.7)	x^2=3.42
No	17(20)	43(64)	P=0.001	5(11)	13(29.3)	P=0.025

DISCUSSION

Several studies have confirmed that *S aureus* is an important cause for both nosocomial and community acquired infections. The nasal culture of this study showed that *S. aureus* is not the only Staphylococcus that is carried by anterior nares. In deed, 50(35.0%) of the 144 cultures, *S.aureus* was isolated together with other Staphylococci and only 35(24.0%) of the 144 cultures were merely *S. aureus*.

S. aureus strains were isolated from 85(59.0%) of the total specimens. This is greater than the range of isolation of *S aureus* from anterior nare (30.0-50.0%) of normal individuals (3). However, compared with intermittent carriage occurring in as much as 90.0% of a sampled population this is not surprising (14). More than 60.0% of the isolates in this study were from surgical and medical wards followed by isolates from gynecology, pediatrics and maternity wards. This is almost twice as prevalent as earlier report from Surgical Department of Tikur Anbessa Hospital in 1983(12). This could be due to the difference in study subjects, inpatients who were hospitalized, used antibiotics, and were with different underlying conditions like diabetes, kidney disease, respiratory disease, liver disease and exposure to *S. aureus* infected subjects in this study group and normal individuals in the earlier study. In addition, it could be due to the abnormal crowdedness of the wards and corridors, as well as the almost continuous use of the operating theatres in this study.

In line with reports from other parts of the world, the majority of the isolates in this study were resistant to the commonly used antimicrobial agents (9,10). About $3/4^{th}$ of the isolates were multiply resistant. This is comparable with different reports on the susceptibility patterns of *S. aureus* (9,15-18). The highest resistance seen with ampicillin could be due to the increased number of β-lactamase producing strains of *S. aureus* and other intrinsic factors. The continuous genetic variation of the strains could also have contributed to the increased

resistance. The sensitivity pattern of *S. aureus* to ampicillin, chloramphenicol, and gentamicin is almost similar to other reports from this region (10). However, isolates of this study showed increased resistance to trimethoprim-salphamethoxazole that could be due to the continuous increment or resistant strains from time to time.

Above 50.0% of the isolates from different wards were found to be resistant to trimethoprim-sulphamethoxazole, oxacillin, chloramphenicol, and ampicillin and below 17.0% were resistant to gentamicin, kanamycin, and clindamycin. Other studies also showed comparable pattern except for clindamycin (10, 12,17,19). Resistance patterns of isolates of this study showed almost four times resistance to chloramphenicol, ten times to erythromycin, five times to kanamycin, seven times to gentamycin, and thirteen times to clindamycin than the isolates from clinical specimens from Addis Ababa in 1991(9). The present isolates showed increased resistance to ampicillin, chloramphenicol, trimethoprim-sulphamethoxazole and erythromycin compared to the 37.0%, 41.0%, 52.0%, and 4.0% reported respectively (20).

The rate of isolation of MRSA in this study was 52.0%, which was higher by 21.0% from the 31.0% reported from clinical specimens from Addis Ababa by 12.0% from the 40.0% reported in 1993 from Jimma, by 44.0% from the 8.0% reported in 1983, and by 11.0% from the 41.0% reported in 1998 (9,11,12, 20). More than 63.0% of the MRSA isolates were from inpatients of surgical ward with different underlying conditions. The finding confirms the observation in previous studies in which *S. aureus* was the most commonly isolated causative agent of hospital acquired sepsis and wound infections (21).

In addition, more than half of the MRSA isolates (56.5%) were from medical ward, which might be due to crowdedness of wards and corridor and the distance of surgical and medical wards that could increase the transmission of *S aureus* via hands of health care workers (12, 22-24).

Antibiograms of 85 isolates of *S aureus* showed higher resistance pattern than that reported by different researchers (9, 12, 20). Multiple resistance of MRSA reported by other studies was also confirmed by this study (8,11,12,18-20,24).

Comparison of variables of Staphylococcal carriers with non-carriers showed significant association with predisposing risk factors ($p<0.05$). The fact that sex and age has no significant association with colonization of *S. aureus* and greater frequency of colonization for boys than for girls was confirmed by this study (25). Previous antibiotic use was associated with higher frequency of colonization followed by possession of known underlying conditions such as diabetes mellitus, respiratory, liver, and kidney disease, as well as previous hospitalization which could have increased the prevalence of MRSA.

Subjects colonized with MRSA were not distinguishable from non-carriers in terms of age (child, $P>0.052$; adults, $P>0.057$) and sex (both, $p>0.1$) and the median duration of hospitalization for MRSA carriers was 21.8 while it is 5.3 for MSSA carriers and 1.2 for non-carriers (data not shown). The prevalence of MRSA among persons with and without predisposing factors was 73.0% and 62.0% respectively. It was found that previous antibiotic use is the major reason for colonization of MRSA.

In general, there is a high rate of nasal carriage of MRSA among inpatients. Hospitalization, previous antibiotic use and underlying conditions are the main predisposing factors for colonization by

MRSA. Most MRSA strains are resistant to other ß-lactam antibiotics. Hence a policy aiming at breaking the transmission route, selection of antibiotics, control measures and guide lines regarding treatment of these isolates is needed, which otherwise incur higher health cost.

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