Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* with Emphasize on Methicilin Resistance with Patients Postoperative and Wound Infections at Yekatit 12 Hospital Medical College in Ethiopia

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Abstract: Background: *Staphylococcus aureus* particularly methicilin resistant *S. aureus* strains are one of the major causes of community and hospital acquired bacterial infections, often causing postoperative and burn wound infections. In-vitro determination of drug resistance patterns of *S. aureus* is critical for the selection of effective drugs for the treatment of staphylococci infections. Objective: The aim of this study was to determine methicillin and antimicrobial susceptibility pattern of *S. aureus* from postoperative and burn wound patients in Ethiopia. Materials and Methods: A cross sectional study was conducted among 378 patients at Yekatit 12 Hospital Medical College from September 2013 to August 2014 in Ethiopia. Swabs from postoperative and burn wound were collected. Swabs were cultured on blood agar and mannitol salt agar and incubated at 35-37°C aerobically for 18-24 hours. Cultures with typical characteristics were identified to *S. aureus* by DNase agar test. *S. aureus* were then screened for MRSA using 30µg cefoxitin disc. The drug susceptibility patterns of *S. aureus* to eleven drugs were determined by disc diffusion procedure and agar dilution for vancomycin. All *S. aureus* isolates examined for beta-lactamase production by employing nitrocefin. Data were analyzed using SPSS version 20 software and logistic regressions were applied to assess any association between dependent and independent variables. P values < 0.05 were taken as statistically significant. Results: Of the 378 swabs analyzed, *S. aureus* was recovered from 179 (47.4%). The prevalence of *S. aureus* was higher in females than males 95(53%) versus 84(47%). Out of 179 *S. aureus* isolates, 67(37.43%) were found out to be MRSA and the remaining 112(62.57%) were MSSA. *S. aureus* isolates were more resistant to penicillin 172(96.1%) and least resistant for vancomycin 10(5.6%) and cephalothin 9(5.0%). MRSA isolates were 67(100%) resistant for penicillin, 63(94.0%) for erythromycin, 62(92.5%) for trimethoprim-sulfamethoxazole and least resistant for cephalothin 9(13.4%) and vancomycin 10(15.0%). Among 179 *S. aureus* isolates, 145(81.0%) were beta-lactamase producers. Furthermore, of 67 MRSA isolates, 61(91.0%) and out of 112 MSSA strains 99(88.4%) were beta-lactamase producers. Conclusion: In this study *S. aureus* isolates exhibited very high degree of resistance to different antibiotics. The prevalence of MRSA was high and this should necessitate caution in prescription of antibiotics without proper indication. The isolates were also multidrug resistant to several combinations of the tested antibiotics. The emergence of vancomycin resistant *S. aureus* highlights the value of prudent prescribing of antibiotics and avoiding their irrational use.

Keywords: Wound Infections, Prevalence, Antimicrobial Susceptibility Pattern, MRSA, Beta-lactamase

1. Introduction

Staphylococcal infections still remain an important cause of mortality and morbidity worldwide despite the development of antimicrobial agents. Among the

staphylococcus species, *Staphylococcus aureus* is the most virulent species of the genus causing both nosocomial and community acquired infections worldwide [1]. The organism has been found to be the most common bacterial agent recovered from blood stream infections, skin and soft tissue

infections, pneumonia and hospital acquired post operative wound infections [2]. Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites. Both acute and chronic wounds are susceptible to contamination and colonization by a wide variety of aerobic and anaerobic microorganisms including *S. aureus* [3].

Changes in the drug susceptibility profile of *S. aureus* have been reported worldwide; thereby making treating infections caused by *S. aureus* more difficult [4, 5]. Dramatic changes in the susceptibility of *S. aureus* to beta-lactam antibiotics particularly to penicillin and cephalosporin in both hospital and community settings have been reported worldwide [6]. The older beta lactams, penicillin and ampicillin are ineffective against more than 80% of the isolated *S. aureus* strains and resistance to many of the non beta lactam agents such as tetracycline, gentamycin, chloramphenicol, erythromycin and clindamycin has gradually increased and reached alarming levels by the 1990s in many parts of the world [7]. Several mechanisms for the development of MRSA have been reported. Among these production of a unique penicillin-binding protein (PBP) that has a low affinity for β -lactam antibiotics and whose effects are determined by several structural genes (*mec*, *mec RI*, *mec I*) [8, 9], production of the usual PBPs, but with modified affinities for the β -lactam drugs, and the production of penicillinase enzyme are the most important ones [10].

In some countries MRSA make up to 75% of all *S. aureus* isolates in hospitals [11]. Prevalence, however, varies markedly in hospitals in the same country and from one country to another. Furthermore, little information exists regarding methicillin and antimicrobial susceptibility pattern of *S. aureus* isolated from wound/pus swabs in Ethiopia. Therefore, this study was designed to evaluate methicillin and antimicrobial susceptibility profile of *S. aureus* isolates from swab samples at Yekatit 12 Hospital, Ethiopia as well as to find out the choice of antimicrobial agent for the treatment of infections due to these organisms in the study area.

2. Materials and Methods

2.1. Study Setting

A facility based cross-sectional study was conducted at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia from September 2013 to August 2014. The hospital is a tertiary level referral and teaching hospital which provides health care services to patients in and around Addis Ababa, the capital city of the country and practical area for medical students.

2.2. Specimen Collection and *S. aureus* Isolation

A total of 378 wound/pus swabs were collected aseptically from patients by employing standard microbiological procedures. The specimens were collected by the attending physician and health officer using sterile applicator stick with cotton swabs moistened with normal saline and test tubes [12]. To minimize contaminating skin commensals, surgical

site and burn wound infections were assessed by senior physicians based on center for disease prevention and control (CDC) criteria. Patients whose diagnosis was confirmed as wound sepsis by a surgeon, pus or serous discharges were aseptically obtained before the wound was cleaned with an antiseptic solution. The socio-demographic variables (age and sex) were taken from each patient through interview. The swabs were then inoculated on blood agar base (Oxoid, Ltd., Basingstoke, and Hampshire, England) and mannitol salt agar (Oxoid, Ltd., Basingstoke, and Hampshire, England) plate and incubated at 37°C for 18-24 hrs. Those colonies that were golden or cream, coagulase positive and that hydrolyzed DNA in DNase agar were considered *S. aureus*.

2.3. Antimicrobial Susceptibility Testing

All *S. aureus* isolates were taken and inoculated on Muller Hinton agar for sensitivity testing according to modified Kirby-Bauer disk-diffusion technique and vancomycin susceptibility was done by agar dilution techniques [13]. Vancomycin resistant strains were also sent to Referral Laboratory for conformation. The following antimicrobials with their concentration in brackets were used: cefoxitin [30 μ g], penicillin [10U], amoxillin-clavunalic acid [30 μ g], trimethoprim-sulfamethoxazole [1.25/23.75 μ g], clindamycin [30 μ g], gentamicin [30 μ g], ciprofloxacin [5 μ g], erythromycin [15 μ g], chloramphenicol [30 μ g], cefuroxime [30 μ g], cephalothin [10 μ g] and vancomycin [30 μ g]. A control reference strain *S. aureus* (ATCC 25923) was used for quality control of culture media and drug disks for susceptibility testing for every batch. This strain was obtained from Ethiopian Public Health Institute (EPHI).

2.4. Beta-lactamase Production Test

All *S. aureus* strains were screened for beta-lactamase production by employing the procedures of Efuntoye et al. [14]. Culture of each isolate was streaked onto sticks impregnated with nitrocefin a chromogenic cephalosporin (Unipath Limited, Hampshire, England) that produces a rapid color change from yellow to pink/red when the beta-lactam ring is hydrolyzed by beta-lactamase. Therefore, production of beta-lactamase was detected by a change in the color of the stick from yellow to red within 10 minutes.

2.5. Ethical Clearance, Data Analysis and Quality Control

The study was conducted after it was ethically reviewed and approved by Ethical Review board of Addis Ababa University and Addis Ababa Health Bureau and permission was obtained from Yekatit 12 Hospital Medical College. Written consents were also obtained from participants. The reliability of the study findings were guaranteed by implementing quality control measures throughout the whole processes of the laboratory work. *S. aureus* ATCC 25923 is a methicillin susceptible strain used to check the conditions were favorable for the detection of resistance carried out in this study. The inoculum size during susceptibility testing was monitored by using 0.5 McFarland standards. All data

from the investigation was coded, double entered and analyzed using SPSS version 20. Descriptive statistics and logistical regressions were used to estimate crude and adjusted odds ratio with 95% confidence interval to the different variables. P-value < 0.05 was considered significant.

Table 1. Distribution of *S. aureus* in study participants with gender and age group at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

		Number of samples	Presence of <i>S. aureus</i>	
		N (%)	Yes (N/%)	No (N/%)
Sex	Male	174 (46.0)	84 (47.0)	90 (45.2)
	Female	204 (54.0)	95 (53.0)	109 (54.8)
	< 1	14 (3.7)	8 (4.4)	6 (3.0)
Age group	1-14	99 (26.2)	39 (21.8)	60 (30.1)
	15-24	89 (23.3)	48 (26.8)	41 (20.6)
	25-34	77 (20.4)	43 (24.0)	34 (17.1)
	35-44	52 (13.8)	24 (13.4)	28 (14.1)
	45-64	35 (9.3)	12 (6.7)	23 (11.6)
	>= 65	12 (3.2)	5 (2.8)	7 (3.5)

Table 2. Association of *Staphylococcus aureus* in study participants with regard to gender and age group at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

		Presence of <i>S. aureus</i>		P-value	COR	95% CI
		Yes (N/%)	No (N/%)			
Sex	Male	84 (47)	90 (45.2)	-	1	-
	Female	95 (53)	109 (54.8)	0.74	0.934	(0.623, 1.40)
	< 1	8 (4.4)	6 (3.0)	0.442	0.542	(0.11, 2.58)
Age group	1-14	39 (21.8)	60 (30.1)	0.849	1.126	(0.33, 3.81)
	15-24	48 (26.8)	41 (20.6)	0.423	0.607	(0.18, 2.06)
	25-34	43 (24.0)	34 (17.1)	0.360	0.562	(0.16, 1.93)
	35-44	24 (13.4)	28 (14.1)	0.790	0.841	(0.23, 3.01)
	45-64	12 (6.7)	23 (11.6)	0.640	0.378	(0.36, 5.28)
	>=65	5 (2.8)	7 (3.5)	-	1	-

COR: crude odds ratio, CI: confidence interval

Figure 1. Methicillin Susceptibility Pattern of *Staphylococcus aureus* at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

3.3. Prevalence of MRSA

Out of 179 *S. aureus* recovered, 67 (37.43%) were found out to be MRSA and the remaining 112 (62.57%) were MSSA (Figure 1). Relatively a higher MRSA were isolated in females than males 37 (55.2%) versus 30 (44.8%) and the highest MRSA was detected in the age group 15-24 years 20 (29.8%) followed by age group 25-34 years 14 (20.9%)

3. Results

3.1. Socio-demographic Distribution of Study Participants

A total of 378, study participants were enrolled in the present study of which 174 (46.0%) were males and 204 (54.0%) females with a sex ratio of 0.91: 1. The ages of study subjects ranged from one month to 88 years with a mean age of 25.7±0.5 years (Table 1).

3.2. Prevalence of *S. aureus* Among Study Participants

Of a total of 378 wound pus swabs, *S. aureus* was isolated from 179 (47.4%). Females had a higher isolation rate of *S. aureus* than males 95 (53.0%) vs 84 (47.0%). Rate of isolation of *S. aureus* was the highest in 15-24 years age group 48 (26.8%) (Table 1). As shown in table 2, the isolation rate of *S. aureus* was not significantly associated with sex [COR, 95% CI: 0.93 (0.62, 1.40), p = 0.74] and age groups (p > 0.05).

and the least in age group less than 1 years 3 (4.5%). No MRSA was isolated in the age group 65 and above years (Table 3). As can be seen in table 3, isolation rate of MRSA in relation to gender was not significantly associated [COR, 95% CI: 1.15 (0.62, 2.11), p = 0.65] as well as any of age groups (p = 0.99).

Table 3. Association of Methicillin Resistant Pattern of *S. aureus* in study participants with gender and age group at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

		MRSA		MSSA		P-value	COR	95% CI
		N	%	N	%			
Sex	Male	30	44.8	54	48.2	-	1	-
	Female	37	55.2	58	51.8	0.656	1.15	(0.625, 2.11)
	< 1	3	4.5	5	4.45	0.999	0.000	-
Age group	1-14	13	19.4	26	23.2	0.999	0.000	-
	15-24	20	29.8	28	25.0	0.999	0.000	-
	25-34	14	20.9	29	25.9	0.999	0.000	-
	35-44	12	18.0	12	10.7	0.999	0.000	-
	45-64	5	7.4	7	6.3	0.999	0.000	-
	>=65	0	0.0	5	4.45	-	1	-

3.4. Antimicrobial Susceptibility Pattern of *Staphylococcus aureus*

Antimicrobial profile of *S. aureus* isolated in this study is presented in table 4 were highly resistant to penicillin 172(96.1%), erythromycin 101(56.4%), trimethoprim-sulphamethoxazole 99(55.3%) and ciprofloxacin 58(32.4%). On contrary, lower resistant was manifested by clindamycin 24(13.4%), amoxillin-clavunic acid 17(9.5%), cefuroxime 16(8.9%), vancomycin 10(5.6%) and cephalothin 9(5.0%) (Table 4).

MRSA isolates were 67(100%) resistant for penicillin, 63(94.0%) for erythromycin, 62(92.5%) for trimethoprim-sulfamethoxazole and least resistant for cephalothin 9(13.4%) and vancomycin 10(15.0%). On the other hand, 105(94.0%) of MSSA were resistant to penicillin. Being resistant/ sensitive for methicillin had a statistically significant chance of being resistant/ sensitive for chloramphenicol, gentamycin and erythromycin [$p < 0.05$] (Table 5).

Table 5. Association of Methicillin Resistant and Sensitive *S. aureus* to different antimicrobial agents at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

Antibiotics []	MRSA (n=67)		MSSA (n=112)		P-value	AOR	95% CI
	N	%	N	%			
AMC ^a [30µg]	15	22.4	2	1.8	0.515	0.410	(0.028, 6.017)
Penicillin G [10U]	67	100	105	94.0	0.999	0.000	-
Vancomycin [30µg]	10	15.0	0	0	0.998	0.000	-
SXT [1.25/23.75µg]	62	92.5	37	33.1	0.136	0.298	(0.061, 1.464)
Chloramphenicol [30µg]	32	47.7	8	7.1	0.001**	4.200	(3.024, 8.411)
Gentamycin [10µg]	43	64.2	11	9.8	0.001**	3.896	(2.035, 6.335)
Cefuroxime [30µg]	16	23.9	0	0	0.999	0.000	-
Clindamycin [30µg]	20	30.0	4	3.6	0.073	8.126	(0.821, 80.41)
Ciprofloxacin [5µg]	42	62.7	16	14.3	0.719	0.808	(0.253, 2.581)
Erythromycin [15µg]	63	94.0	38	34.0	0.001**	3.044	(6.005, 11.224)
Cephalothin [10µg]	9	13.4	0	0	-	1	-

AMC^a-Amoxillin-clavunalic acid, ** Significant at P value < 0.05

3.5. Beta-lactamase Production in *Staphylococcus aureus*

Of 179 *S. aureus* isolates, 145(81.0%) were beta-lactamase producers. Furthermore, of 67 MRSA isolates 61(91.0%) and out of 112 MSSA strains 99 (88.4%) were produced beta-lactamase enzyme.

4. Discussion

The overall *S. aureus* isolation rate in this hospital was 47.4% which is comparable with the rate reported in other similar studies in Ethiopia [15, 16], but it is higher than rates observed in Uganda and Indian [17, 18]. In contrast higher rate of isolation was reported in Nigeria [19] and United States [20, 21]. The incidence of *S. aureus* isolation was more common in females 53.0% than in males 47.0%. This is in agreement with previous studies done in Ethiopia [15, 16]. The prevalence of MRSA was found out to be 37.43% and the remaining 62.57% were MSSA. This finding is in agreement with similar studies in Ethiopia [22], India [2] and Uganda [17], but this study was less than that had been reported in other studies in Ethiopia [16, 23, 24], Sudan [25],

Table 4. Antimicrobial Susceptibility Pattern of *S. aureus* strains to different antimicrobial Agents at Yekatit 12 Hospital Medical College from September 2013 to August 2014, Addis Ababa, Ethiopia.

Antibiotics []	Resistant		Sensitive	
	N	%	N	%
Cefoxitin [30µg]	67	37.4	112	62.6
Amoxillin-clavunalic acid [30µg]	17	9.5	162	90.5
Penicillin G [10U]	172	96.1	7	3.9
Vancomycin [30µg]	10	5.6	169	94.4
SXT ^a [1.25/23.75µg]	99	55.3	80	44.7
Chloramphenicol [30µg]	40	22.3	139	77.7
Gentamycin [10µg]	54	30.2	125	69.8
Cefuroxime [30µg]	16	8.9	163	91.1
Clindamycin [30µg]	24	13.4	155	86.6
Ciprofloxacin [5µg]	58	32.4	121	67.6
Cephalothin [10µg]	9	5.0	170	95.0
Erythromycin [15µg]	101	56.4	78	43.6

SXT^a= Trimethoprim-Sulfamethoxazole

Nigeria [26] and United States [20]. This variation in the prevalence rates of MRSA in the same country or between countries could be attributed by length of study period, number of study sites, sample size, sample type and the laboratory procedures employed. Prevalence of MRSA in the present study, however, did not vary significantly by gender ($p=0.65$) and age group ($p=0.99$) and this is in agreement with earlier reports in Ethiopia [22] indicating that gender and age are not risk factor for the acquisition of MRSA. MRSA on surgical wards is becoming increasingly common especially in critically ill patients who have spent prolonged periods on the intensive care unit [27].

Antimicrobial susceptibility test on 179 *S. aureus* isolates against eleven commonly used antibiotics indicated that 96.1% were resistant to penicillin, 56.4% to erythromycin, 55.3% to trimethoprim-sulphamethoxazole and this finding is in agreement with previous findings in different parts of Ethiopia [16, 23, 24], Nigeria [26], Sudan [25] and India [28]. Moderate sensitivity was seen in ciprofloxacin 32.4% and gentamicin 30.2% which is consistent with studies in Uganda, Nigeria and Karachi [17, 26, 29]. The lowest drug resistant was observed for vancomycin 5.6% and cephalothin

5.0%. All MRSA isolates in this study were completely resistant (100%) to penicillin. Similar results were observed among MRSA strains in India [28] and Spain [27]. Unlike most studies in Ethiopia [22] and elsewhere in the world [17, 27] vancomycin resistant was very high in the current study. Ten (5.6%) out of 179 *S. aureus* isolates were resistant to vancomycin and of 67 MRSA, 15% were vancomycin resistant. MRSA that are also resistant to vancomycin was ranged from 0% in Ethiopia [22], Karachi [29] and Uganda [17] to 8% in Iran [30] and Malaysia [31] have been reported. A similar result was obtained for vancomycin in previous reports in Ethiopia [16, 17, 24], Nigeria [26] and Tehran [32]. Treatment failure has been incriminated as a cause of decreased susceptibility of staphylococci to vancomycin [33]. Wise use and continuous surveillance susceptibility testing of MRSA against vancomycin have been reported as a remedy to control reduced susceptibility of staphylococci to vancomycin [34, 35].

In the present study, all *S. aureus* isolates were tested for beta-lactamase production. It has been shown that out of 179 isolates, 81.0% were beta-lactamase producers. Beta-lactamase producing strains of *S. aureus* in the present study were consistent with reported in Nigeria [14], but much higher than that has been reported in previous study in Ethiopia [22]. The study further depicted that out of 67 MRSA strains 91.0% were produced beta-lactamase enzyme which was higher than a study in India [36] and out of 112 MSSA strains 88.4% were found out to be beta-lactamase producers.

5. Conclusion

In conclusion, *S. aureus* was the most prevalent bacteria among others isolated from postoperative and burn wounds. These *S. aureus* were also exhibited very high degree of resistance to different antibiotics. The prevalence of MRSA was high and should necessitate caution in prescription of antibiotics without proper indication. The isolates were also multidrug resistant to several combinations of the tested antibiotics. The emergence of vancomycin resistant *S. aureus* highlights the value of prudent prescribing of antibiotics and avoiding their irrational use. Such occurrence of vancomycin resistant *Staphylococcus aureus* is worrisome to physicians as it is currently the main antimicrobial agent to treat infections with MRSA. Antimicrobials such as clindamycin, amoxicillin-clavulanic acid, cefuroxime, vancomycin and cephalothin are recommended for empirical treatment for *S. aureus* infection in areas with low or no antimicrobial susceptibility testing facilities. Further, molecular techniques are needed to identify strain typing of *S. aureus* and resistant genes.

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