

Prevalence of methicillin resistant *Staphylococcus aureus* in patient specimens referred to National Reference Laboratory, Ethiopian Public Health Institute

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Abstract

Introduction: *Staphylococcus aureus* is the leading cause of increased morbidity and mortality both in hospital and community settings and produces a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia.

Objective: To determine the prevalence and antibiotic pattern of methicillin resistant *Staphylococcus aureus* and methicillin sensitive *Staphylococcus aureus* among referred specimens.

Materials and Methods: Cross sectional study design of modified Kirby Bauer disk diffusion and minimum inhibitory concentration was conducted at Ethiopian Public Health Institute National Referral bacteriology Laboratory. All types of specimens those collected from 2008 to 2014 period were included in the study, and 238 *Staphylococci* isolates were identified and subject to E-test to determine their MRSA status.

Results: A total of 2,768 *Staphylococcus aureus* isolates were identified from a range of clinical specimens and *S. aureus* accounted for 75.9% (2102) from all *Staphylococcus* species and 50.5% from the 4,159 identified bacterial pathogens. The prevalence of Methicillin resistance *Staphylococcus aureus* was 44.5% (106/238) and that of Methicillin sensitive *Staphylococcus aureus* was 55.5 % (132/238). From the total methicillin resistant *Staphylococcus aureus* isolates (n=106), 49 of them showed highest level of resistance with concentration of ceftiofur 24 and above mg/ml. The highest proportion of methicillin resistant *Staphylococcus aureus* was found in age group 19+ years (75.5%) followed by age group 6-18(18.9%).

Conclusion and Recommendation

Among all bacterial pathogens, *Staphylococcus aureus* accounted for 50.5% which indicated that the pathogen was the leading cause of clinical infection among the various bacterial agents. The highest prevalence of methicillin resistant *Staphylococcus aureus* and multidrug resistant strains of *Staphylococcus aureus* were isolated from clinical specimens which was found to be alarming. Therefore, strengthening and expanding the existing antimicrobial resistance surveillance system is recommended for effective infection prevention and control operations at health facilities.

Introduction

Methicillin resistance *Staphylococcus*(S) species is resistance to methicillin antibiotics by new gene of Mec A located on *staphylococcal* cassette chromosome. This new penicillin binding protein 2a (PBP) has a very low affinity for β -lactam antibiotics and this strain become resistance phenotypically (Klevens et al. 2006; Moran et al. 2006).

Various studies have recognized methicillin resistant *Staphylococcus aureus* (MRSA) strains that appear to be well adapted to the hospital environment (Enright et al. 2002). They are frequently isolated from bacteremia, wound infections and pneumonia (Millar et al. 2007). Transmission of MRSA in the hospital setting occurs via person-to-person spread that involves health-care staff hands and also transmission from hospital environment to patients (Millar et al. 2007). Available evidences show that hospital-acquired MRSA infection increases risks of morbidity,

mortality and also costs of medication (Cosgrove et al. 2005). A more virulent and transmissible version of hospital associated MRSA is community associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) that can be evident in individuals in the community who do not have the risk factors for the hospital associated MRSA (Boucher and Corey 2008). These new strains of *S. aureus* exhibiting distinctive combinations of virulence factors and resistance traits have been linked with increased morbidity and mortality in the community (Frank et al. 2010). The mobile genomic island, *staphylococcal* cassette chromosome *mec* (SCC*mec*) harbors the *mecA* gene which confer the isolates with an additional penicillin-binding protein (PBP2A) that has reduced affinity of β -lactam antibiotics (Ito et al. 2003). Shortly after the antibiotic methicillin was made available in markets in 1960; methicillin resistant isolates were reported after the screening of clinical isolates in England (Jevons 1961). By the year 1967, MRSA was reported from Switzerland, France, Denmark, Australia and India

(Parker and Hewitt, 1970). In the early 1980s, several countries reported multidrug resistant MRSA (Boyce et al. 1983). Up till now, MRSA is the most frequently identified antibiotic-resistant pathogen in many parts of the world, including Europe, the Americas, North Africa, Middle East and East Asia. It is a common hospital pathogen that causes severe infections and complications because of its multiple antibiotic resistance traits (Kluitmans et al. 1997). The rates at which MRSA isolates have been recovered was increasing worldwide over the past decades, as data from continuing surveillance initiatives such as the National Nosocomial Infection Surveillance System (NNIS) and European Antimicrobial Resistance Surveillance System shows (Fridkin et al. 2002; Tiemersma et al. 2004). Different countries reported different rates of MRSA prevalence. It was observed that MRSA prevalence was 23% in Australia, 67% in Japan, 40% in South Pacific, 32% in USA, and 26% in Europe (Diekema et al. 2001). In addition, MRSA was reported to account for >60% of *S. aureus* isolates in the U.S. Hospital Intensive care Units (ICUs) (NNIS 2004).

The limited information available in Africa indicates that MRSA is reported at different rates in different parts of the continent (Maina et al. 2012; Sala et al. 2012). In Ethiopia, national MRSA surveillance system to determine the magnitude of the problem and information regarding the prevalence of MRSA is limited. However, there are some studies done at institution level that reported higher rate of MRSA in different hospitals (Tekalign and Ketema 2013; Aguma et al. 2013). Despite the existence of patchy report on MRSA in Ethiopia, more studies need to be done in order to have comprehensive figure on the overall burden of MRSA in the Ethiopian context. Therefore, this study was aimed to determine the magnitude and pattern of MRSA in clinical specimens referred to national bacteriology and mycology reference laboratory (NRL) of the Ethiopian Public Health Institute (EPHI).

Materials and Methods

A cross sectional study design was conducted at EPHI from February to May 2016 on all patients' specimens referred to the NRL. All *Staphylococci* isolates identified from all types of specimens from 2008 to 2014 were included in the study. *Staphylococcus aureus* isolates identified from other than referral linkage like isolates sent for confirmation and isolated from project samples were excluded from the study. Data were collected and then entered into Epi Info (version 3.5.4.) then exported and cleaned using excel sheet and analyzed using SPSS statistical software

version 21.0 (IBM Armonk, NY). Ethical approval was obtained from Scientific and Ethical Review Office of EPHI.

Laboratory Techniques: Conventional culture technique was used for isolation of bacterial pathogen. Blood agar plates supplemented with 5% sheep blood and mannitol salt agar plates were used to identify organisms causing the infection (Difco, Detroit, Mich, USA). All culture plates were incubated at 35 ± 2 for about 24hrs and examined for bacterial growth. Morphological analysis of bacterial colony, beta hemolytic, creamy white colonies on blood agar, growth and color on mannitol salt agar were used to isolate the *Staphylococci* organism. Gram stain, catalase, coagulase and DNase tests techniques were employed to confirm the staphylococci species. The standard Kirby Bauer disk diffusion was used to do AST. Briefly, standard inoculums *S. aureus* isolates plated on Muller Hinton agar for sensitivity testing according to modified Kirby-Bauer disk-diffusion technique and E-strips test using Cefoxitin (30µg), Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg), Tetracycline (30µg) and Gentamycin (10µg) was used to determine the MRSA status of 238 *S. aureus* isolates. Control reference strain of *S. aureus* (ATCC) was used for quality control of culture media, drug disks for susceptibility testing and E- strip test for every laboratory process. CLSI guideline (CLSI, 2017) was used to determine the susceptibility pattern and result interpretation of antimicrobial drugs tested.

Result

Frequency of Bacterial Isolation: Out of the 4159 identified bacterial pathogens, 66.6% (2,768) of them were *Staphylococcus* species. About 51% (2116) of the isolates were from males. *Staphylococci* was the number one leading bacterial pathogen detected over the years followed by *Escherichia coli* (*E. coli*). *S. aureus* accounted for 75.9% while the remaining 24.1% were coagulase negative *Staphylococci*.

Occurrence of MRSA isolates: Antimicrobial susceptibility testing was conducted on 238 *S. aureus* isolates (pusy discharge from other sites: n=136; blood: n=4; ear discharge: n=55; nasal: n=39 and eye: n=4). To determine their MRSA status, the isolates were tested using cefoxitin (30mg). The prevalence of MRSA was 44.5% (106/238) and that of MSSA was 55.5 % (132/238) (Table-1). From the total MRSA isolates (n=106), 49 of them showed highest level of resistance with concentration of cefoxitin 24 and above mg/ml. The highest proportion of MRSA was found in age group 19 years and above (75.5%) followed by age group 6-18(18.9%).

Table 1: Age and sex distribution of MSSA and MRSA from referred clinical patient, Ethiopia, 2008-2014

Sex	Cefoxitin E-test profile of <i>S. aureus</i>		Total
	MSSA*	MRSA**	
Sex			
Male	70 (53.3%)	52 (49.1%)	122(51.3%)
Female	62(47.0%)	54 (50.9%)	116(48.7%)
Age (Years)			
1-5	3(2.3%)	6(5.75)	9(3.8%)
6-18	23(17.4%)	20(18.9%)	43(18%)
≥ 19	106(80.3%)	80(75.5%)	186(78.2%)
Total	132(55.5%)	106(44.5%)	238 (100%)

*Methicillin susceptible *Staphylococcus aureus*; **Methicillin Resistant *Staphylococcus aureus*

MRSA and MSSA in different clinical samples: The highest rate of MRSA was detected from pus discharge from other sites specimen 55.7% (59) followed by ear 24.5% (26) and nasal 17.9% (19). Comparing MRSA proportion within the same specimen shows that MRSA was highest for *S. aureus* recovered from blood (2 out of 4) followed by nasal specimen (19 out of 39); ear discharge (26 out of 55); pus (59 out of 136) and none of *S. aureus* from eye infection were MRSA.

Susceptibility of *S. aureus* to other antimicrobials:

The isolates were also tested against penicillin (10U), erythromycin (15mg), clindamycin (2mg), tetracycline (30mg) and gentamycin (10mg). The result of minimum inhibitory concentration (MIC) test

showed that *S. aureus* were resistant to penicillin, erythromycin and clindamycin at the rate of 86.5%, 76.9% and 56.3%, respectively. On the other hand, resistance level to tetracycline, gentamycin and inducible clindamycin were below 50% (36.6%, 4.2% and 25.6% respectively). The proportion of *S. aureus* isolates that were intermediate resistant for tetracycline was higher compared to the sensitive and resistant strains (Table-2). Of the total *S. aureus* isolates, MIC results gave higher proportion rate of multidrug resistant with E-test accounting for 22.5% for two drugs, 18.9% for three drugs and 9.2% for four drugs while disk diffusion gave the proportion 5.6%, 1.5% and 1.1% in for two, three and four drugs, respectively (Table 3).

Table 2: Antimicrobial susceptibility pattern of *S. aureus* recovered from specimens of the national referral laboratory, Ethiopia, 2008-2014

Resistance level	Tested antimicrobial panel						
	FOX(30)	PEN(10)	ERY(15)	CLI(2)	TET(30)	GEN(10)	D-ICLI
S	55.5%(132)	13.5%(32)	23.1%(55)	43.7%(104)	26.1%(62)	92.0%(219)	74.4%(177)
R	45.5%(106)	86.5%(206)	76.9%(183)	56.3%(134)	36.6%(87)	4.2%(10)	25.6%(61)
I	0	0	0	0	37.4%(89)	3.8%(9)	0
Total (n=238)							

FOX=cefoxitin; PEN=penicillin; ERY=erythromycin; CLI=clindamycin; TET=tetracycline; GEN=gentamycin; D-ICLI=inducible clindamycin

Table 3: Antimicrobial resistance profile of multidrug resistant *S. aureus* isolates recovered from specimens referred to national reference laboratory, Ethiopia, 2008-2014

Antibiogram	%(N)
Cefoxitin + Erythromycin	34.0(81)
Cefoxitin + Clindamycin	25.6(61)
Cefoxitin + Tetracycline	21.4(51)
Cefoxitin + Gentamycin	0.4(1)
Cefoxitin + Penicillin	40.8(97)
Penicillin + Erythromycin	68.1(162)
Penicillin + Clindamycin	48.3(115)
Penicillin + Tetracycline	36.6(87)
Penicillin + Gentamycin	4.2(10)
Erythromycin + Tetracycline	24(57)
Erythromycin + Gentamycin	2.1(5)
Clindamycin + Tetracycline	21.9(52)
Clindamycin + Gentamycin	3.8(9)
Tetracycline + Gentamycin	0.4(1)
Cefoxitin + Erythromycin + Clindamycin	18.9(45)
Cefoxitin+ Erythromycin + Clindamycin + Tetracycline	9.2(22)

Discussion

The overall *S. aureus* identification rate for this study was 50.5% which is very high and comparable with previous studies conducted in Ethiopia (Agumas et al. 2013; Tebelay and Adane 2016). However, the current finding is lower than the report from studies conducted in Africa and elsewhere (Moran et al. 2006; Salah et al. 2012; Maina et al. 2013).

In the present study, the prevalence of MRSA was 44.5% which is higher than report from previous retrospective study conducted at this national reference laboratory before two decade (which was 30.5%) (Abera et al. 1991). The discrepancy can be explained by the techniques and the antibiotic used formerly. Nowadays, evidences suggest that the use of cefoxitin as a surrogate to oxacillin can best detect MRSA than the use of oxacillin which was the common practice back then (Derek et al. 2005). Nonetheless, the prevalence of MRSA reported in the current study is much lower than a report from Kenya (Maina et al. 2013) and Sudan (Elhassan et al. 2015).

The prevalence of MRSA was slightly higher in female than males which is similar with previous study conducted in Ethiopia (Abera et al. 1991; Tebelay et al. 2016). In addition, the distribution of MRSA was higher in adults than children aged between 1-18 years of age which is in agreement with other study done in Ethiopia (Tebelay et al. 2016).

The resistance levels of *S. aureus* for penicillin, erythromycin, clindamycin, tetracycline and gentamycin were higher compared to studies conducted in Addis Ababa (Abera et al. 1991) and Mekelle hospital (Zeamanual et al. 2009). Compared to MSSA, MRSA showed higher antimicrobial resistance proportion for range of tested antimicrobials (penicillin, tetracycline, clindamycin and inducible clindamycin). Similar trend was also reported Iran (Solmaz et al. 2014).

In the present study, the result showed that the prevalence of multidrug resistant MRSA is lower than other studies conducted in Ethiopia (Tekalign et al. 2013; Abera et al. 1991; Tebelay et al. 2016) and elsewhere (Ito et al. 2001; Zeamanual et al. 2009). All these variations of antimicrobial resistance prevalence with present study and others may be due to various reasons like sensitivity of techniques employed, study period and procedure followed in microbiology laboratory like suspension preparation and incubation condition (Derek et al. 2005).

Conclusions and Recommendations

The overall *S. aureus* prevalence was 50.5% which indicate that the pathogen was the lead cause of

clinical infection among the various bacterial agents. The high prevalence of MRSA and multidrug resistant strains of *S. aureus* isolated from clinical specimens is found to be alarming. Therefore, strengthening and expanding the existing AMR surveillance system is recommended for effective infection prevention and control activities of health facilities.

Conflict of interest: Authors declare no conflict of interest.

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