Original article

Methicillin-resistant *Staphylococcus aureus* with genotyping method among human immunodeficiency virus positive pediatric patients in Northwest Ethiopia: A cross-sectional study design

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Abstract

Background: Increasing evidence suggests that methicillin-resistant *Staphylococcus aureus* (MRSA) infections are becoming more prevalent throughout the human immunodeficiency virus (HIV) infected community. However, there is scarcity of data about the prevalence of MRSA among HIV positive pediatric patients in the study area.

Objectives: To determine the prevalence and types of MRSA among *S. aureus* isolates of HIV positive pediatric patients in the Amhara National Regional State, Northwest Ethiopia.

Methods: Pediatric patients who attended the clinic from December 2013 to April 2014 were included in the study. Genotype MRSA VER 3.0 was used for characterization of *S. aureus* isolates. This detected methicillin-resistance-mediating mecA and mecC genes and the bicomponent cytotoxic virulence factor Panton–Valentine leukocidin (PVL). Data were analyzed using SPSS version 20.

Results: Among 126 *S. aureus* isolates, 37.3% and 11.9% were mecA and Panton–Valentine leukocidin gene positive, respectively. Patients of FHRH (P = 0.04) and DRH (P = 0.02) have statistical significance for mecA gene. Panton–Valentine leukocidin gene positive strains were about 97% less likelihood to be mecA gene positive (P = 0.001).

Conclusion: A high prevalence of pathogenic MRSA strains among HIV positive pediatric patients was observed. Most of the MRSA types were hospital acquired. Hence, strict hygienic approaches by healthcare workers in hospitals should be implemented. In addition, screening and treatment of MRSA for HIV positive pediatric patients is recommended. [*Ethiop. J. Health Dev.* 2018;32(3):00-000]

Key words: MRSA, pediatrics, HIV, Ethiopia

Introduction

The increasing frequency of antimicrobial resistance among infectious organisms is of great concern to both the general public and health service providers (1). The acquisitions of the staphylococcal cassette chromosome harbor the mecA gene. Expression of mecA gene yields a penicillin-binding protein (PBP2a) that reduces the bacterium's susceptibility to beta-lactam drugs. It is interesting to note that the cassette that harbors the mecA gene is also capable of 'trapping' drug-resistant plasmids and transposons, and thereby increasing the drug resistance of *S. aureus* (2).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is contagious and can cause life-threatening infection. Although MRSA was originally associated with health care-acquired infections, it also began to be recognized as an important cause of community-onset infections in the late 1990s (3-5). Community-acquired MRSA (CA-MRSA) isolates carry much smaller staphylococcal cassettes (SCCmec types IV, V or VII), (6,7) have different lineages (8-12) and carry the Panton–Valentine leukocidin (PVL) gene (8). Innate immunity represents the main host defense against *S. aureus*, with neutrophils being the primary cellular defense of the innate immune response (13,14). In people infected with HIV, *S. aureus* infections account for significant morbidity (15-17). A higher isolation rate of MRSA has been reported from most African countries, including Nigeria (29.6%), Kenya (27.7%), Cameroon (21.3%), Côte d'Ivoire (16.8%) and Morocco (14.4%) (18).

MRSA is an important cause of infections and HIV-infected individuals are frequently susceptible to this pathogen. HIV positive pediatric patients are also the most vulnerable group of people for different types of infectious disease. In Ethiopia, particularly in Amhara Region, there is a paucity of data about the prevalence of MRSA among HIV positive pediatric patients. Moreover, we haven't seen any data on genotype level to assess the real burden of MRSA on this population group. Hence, this study was designed to determine the prevalence and type of MRSA with genotype method among *S. aureus* isolates of HIV positive pediatric patients in Northwest Ethiopia. The findings of this research, especially the identification of types of

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MRSA, will have great public health implications for the prevention and control of MRSA in Ethiopia.

Materials and methods

Study design and period: An institutional-based cross-sectional study was conducted from December 2013 to April 2014. Study participants were recruited from the pediatric HIV clinics at Felege Hiwot Referral Hospital (FHRH), Debre-Tabor Hospital (DTH) and Dessie Referral Hospital (DRH), in the Amhara National Regional State. Participants living with HIV, <18 years of age, receiving medical care at the aforementioned health facilities, were included in the study. Patients who were on antibiotic treatment for any bacterial infection during the time of data collection were excluded from the study.

Variables: Sex, age and residency were used as the demographic variables. Medical history (WHO clinical categories, immunological markers and type of ARV drugs used during therapy, and so on), were also used as clinical variables, which were taken from the medical records of clients at the aforementioned hospitals. The MRSA mediating mecA and mecC genes and the bicomponent cytotoxic virulence factor PVL genes were also considered as variables.

Data collection: A structured questionnaire was used to collect data on patients' demographic characteristics. Moreover, patients' medical records were used to assess the clinical history of the participants. Genotypic data on *S. aureus* colonization and drug susceptibility profiles of the isolates were also collected from the genotype MRSA test.

Laboratory procedures: From each participant, specimens for *S. aureus* isolation were collected from the anterior nares, the back of the wrist and the perineum using sterile swabs. Swabs were plated on mannitol salt agar and blood agar. Culture plates were incubated at 35ºC for 24 to 48 hours aerobically. The 202 culture positive patients were prepared for further molecular detection of *S. aureus* and MRSA.

Detection of S. aureus and MRSA by Genotype MRSA VER 3.0: The Genotype MRSA VER 3.0 is a qualitative *in vitro* test for characterization of *S. aureus* and *S. epidermidis* strains. At the same time, the methicillin-resistance-mediating mecA and mecC genes, as well as the bicomponent cytotoxic virulence factor PVL, were detected. The Genotype MRSA test is based on the DNA •STRIP technology.

DNA extraction, amplification and hybridization: Bacteria freshly grown on culture plates were used as starting material for DNA extraction. The standardized quick protocols were used for DNA extraction from cultured material. After spin down for 5 minutes at maximum speed, 5µl of the supernatant was collected for polymerase chain reaction (PCR). All reagents needed for amplification, such as polymerase and primers, were included in the amplification mixes. The thermal cycler was installed based on the Hain Lifescience Protocol 'MRSA HOT'. We performed hybridization on 'overview equipment programs', which are available on www.hain-lifescience.com.

Data quality assurance: Pre-trained antiretroviral treatment (ART) nurses collected samples and demographic and clinical data. Culture was done by experienced bench microbiologists. Here, we were adhering strictly to the established procedures of the test, according to the 'Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus*' (19). Moreover, data were double entered and analyzed in consultation with a statistician.

Definitions: Participants were classified as 'MRSA colonized' if mecA gene was detected from either of the nares, premium or the skin. Participants colonized with both methicillin-susceptible *S. aureus* (MSSA) and MRSA (regardless of site) were classified as 'MRSA colonized'.

Statistical methods: Data were entered and analyzed using SPSS statistical package, version 20. Binary logistic regression analysis was carried out to determine the association between explanatory variables and the outcome variable. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated, and the results were considered statistically significant at $p < 0.05$.

Results

Socio-demographic characteristics: In this study, 400 HIV-infected pediatric patients were included. Of those, 202 were phenotypically isolated as culture positive for *S. aureus* and their details have been discussed elsewhere (20). However, among 202 culture positive samples, only 126 were eligible and confirmed with genotype MRSA. Most of the participants (78.6%) were urban dwellers and the majorities were from Felege Hiwot Referral Hospital (60.3%). Females accounted for 54% of the cases (Male: Female ratio = 1.17:1) with the mean age of 10.00 (SD ±3.34). The majority of the participants (81.0%) were on ART (Table 1).

Table 1: **Demography and general description of variables among HIV positive pediatric patients who were isolated as *S. aureus* with genotype MRSA in Northwest Ethiopia**

Variables	Frequency no. (%)
Study sites	
FHRH	76 (60.3)
DRH	43 (34.1)
DTH	7 (5.6)
Gender	
Female	68 (54.0)
Male	58 (46.0)
Residence	
Urban	99 (78.6)
Rural	27 (21.4)
Hospitalization in the last one year	
Yes	5 (4.0)
No	121 (96.0)
Skin lesion	
Yes	13 (10.3)
No	113 (89.7)
Taking ART	
Yes	102 (81.0)
No	24 (19.0)
Age	
<5	11 (8.7)
≥5	115 (91.3)
Last CD4 count	
≤403	35 (27.8)
404-638	31 (24.6)
639-918	28 (22.2)
>918	32 (25.4)
mecA gene	
Positive	47 (37.3)
Negative	79 (62.7)
mecC gene	
Positive	2 (1.6)
Negative	124 (98.4)
PVL gene	
Positive	15 (11.9)
Negative	111 (88.1)

Key: FHRH = Felege Hiwot Referral Hospital, DRH = Dessie Referral Hospital, DTH = Debre-Tabor Hospital

Prevalence and associated risk factors of MRSA: Among *S. aureus* positive patients, 47 (37.3%) were mecA gene positive, as computed from counts of MRSA at any one of the specimens collected from each patient. The majority of MRSA cases were identified among those who had a history of hospitalization in the last 12 months (80.0%). Moreover, patients with the last CD4 count less than or equal to 403 were the highest group for MRSA colonization (54.3%). The highest proportion of MRSA species were identified from the perineum (59.5%), followed by skin swab (43.6%) (Figure 1). From the chi- squared test, the variables such as study sites ($\chi 2 = 8.17$), history of hospitalization in the last one year ($\chi 2 = 4.05$), last CD4 count ($\chi 2 = 6.12$) and PVL gene expression ($\chi 2 = 22.84$) showed an association to mecA gene (Table 2). However, with the logistic regression model, being patients of FHRH (AOR = 19.6, 95% CI: 2.20-175.49, P value = 0.04) and DRH (AOR = 13.6, 95% CI: 1.46-126.15), P value = 0.02) had statistical significance for the positivity of mecA gene. Furthermore, PVL gene positive strains were about 97% less likelihood to be mecA gene positive (AOR = 0.03, 95% CI: 0.003-0.19, P value = 0.001) (Table 3).

Table 2: **The chi-squared values of variables with mecA gene among *S. aureus* isolates of HIV positive pediatric patients in Northwest Ethiopia**

Variables	mecA gene		Chi square
	Positive	Negative	
Study sites			$\chi2 = 8.17$ Df = 2
FHRH	24 (31.6)	52 (68.4)	
DRH	17 (39.5)	26 (60.5)	
DTH	6 (85.7)	1 (14.3)	
Gender			$\chi2 = 0.26$ Df = 1
Female	24 (35.3)	44 (64.7)	
Male	23 (39.7)	35 (60.3)	
Residence			$\chi2 = 0.17$ Df = 1
Urban	36 (36.4)	63 (63.6)	
Rural	11 (40.7)	16 (59.3)	
Hospitalization in the last one year			$\chi2 = 4.05$ Df = 1
Yes	4 (80.0)	1 (20.0)	
No	43 (35.5)	78 (64.5)	
Skin lesion			$\chi2 = 0.26$ Df = 1
Yes	4 (30.8)	9 (69.2)	
No	43 (38.1)	70 (61.9)	
Taking ART			$\chi2 = 0.00$ Df = 1
Yes	38 (37.3)	64 (62.7)	
No	9 (37.5)	15 (62.5)	
Age			$\chi2 = 0.34$ Df = 1
<5	5 (45.5)	6 (54.5)	
≥5	42 (36.5)	73 (63.5)	
Last CD4 count			$\chi2 = 6.12$ Df = 3
≤403	19 (54.3)	16 (45.7)	
404-638	10 (32.3)	21 (67.7)	
639-918	9 (32.1)	19 (67.9)	
>918	9 (28.1)	23 (71.9)	
mecC gene			$\chi2 = 0.14$ Df = 1
Positive	1 (50.0)	1 (50.0)	
Negative	46 (37.1)	78 (62.9)	
PVL gene			$\chi2 = 22.86$ Df = 1
Positive	14 (93.3)	1 (6.7)	
Negative	33 (29.7)	78 (70.3)	

Key: $\chi2$ = Chi square, Df = Degree of freedom

Table 3: **Association of variables with mecA gene among *S. aureus* isolates of HIV positive pediatric patients in Northwest Ethiopia**

Variables	mecA gene		COR (95%CI),PV	AOR (95% CI),PV
	Positive	Negative		
Study sites				
FHRH	24 (31.6)	52 (68.4)	13.0(1.48-114.03),0.02	19.6(2.20-175.49),0.08
DRH	17 (39.5)	26 (60.5)	9.1(1.01-83.10),0.05	13.6(1.46-126.15),0.02
DTH	6 (85.7)	1 (14.3)	1	1
Gender				
Female	24 (35.3)	44 (64.7)	1.2(0.58-2.48),0.61	1.74(0.73-4.20),0.21
Male	23 (39.7)	35 (60.3)	1	1
Residence				
Urban	36 (36.4)	63 (63.6)	1.2(0.50-2.87),0.67	1.45(0.53-3.94),0.46
Rural	11 (40.7)	16 (59.3)	1	1
Hospitalization in the last one year				
Yes	4 (80.0)	1 (20.0)	0.14(0.01-1.27),0.08	0.41(0.02-6.36),0.53
No	43 (35.5)	78 (64.5)	1	1
Skin lesion				
Yes	4 (30.8)	9 (69.2)	1.38(0.40-4.76),0.61	2.40(0.38-14.95),0.34
No	43 (38.1)	70 (61.9)	1	1
Taking ART				
Yes	38 (37.3)	64 (62.7)	1.01(0.40-2.53),0.98	1.22(0.38-3.91),0.72
No	9 (37.5)	15 (62.5)	1	1
Age				
<5	5 (45.5)	6 (54.5)	1.44(0.42-5.03),0.56	0.55(0.13-2.36),0.42
≥5	42 (36.5)	73 (63.5)	1	1
Last CD4 count				
≤403	19 (54.3)	16 (45.7)	0.33(0.12-0.91),0.03	0.49(0.14-1.72),0.26
404-638	10 (32.3)	21 (67.7)	0.82(0.28-2.41),0.72	1.05(0.28-3.87),0.94
639-918	9 (32.1)	19 (67.9)	0.82(0.27-2.49),0.73	0.81(0.23-2.75),0.73
>918	9 (28.1)	23 (71.9)	1	1
mecC gene				
Positive	1 (50.0)	1 (50.0)	0.60(0.03-9.65),0.71	1.69(0.02-105.72),0.80
Negative	46 (37.1)	78 (62.9)	1	1
PVL gene				
Positive	14 (93.3)	1 (6.7)	0.03(0.004-0.23),0.001	0.03(0.003-0.19),0.001
Negative	33 (29.7)	78 (70.3)	1	1

Key: COR = Crude Odds Ratio, AOR = Adjusted Odds Ratio, CI = Confidence Interval, PV = P-value, FHRH = Felege Hiwot Referral Hospital, DRH = Dessie Referral Hospital, DTH = Debre-Tabor Hospital

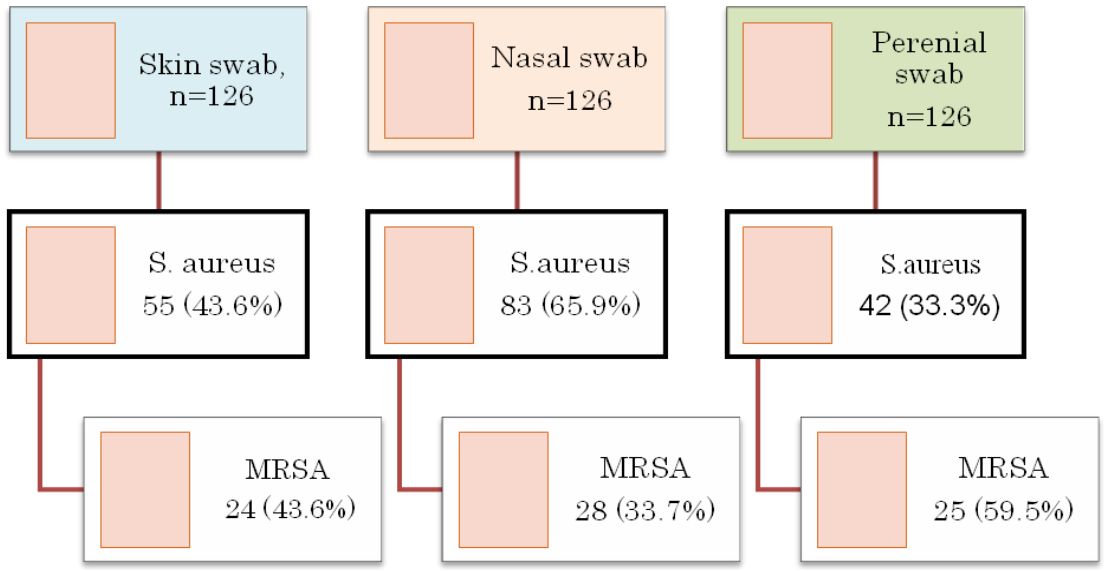

Figure 1: **Proportion of *S. aureus* and MRSA isolates by specimen type among HIV-infected children in Amhara National Regional State**

Discussion

In the current study, a prevalence of MRSA colonization was 37.3%, as computed from counts of MRSA at any one of the specimens collected from each patient. This finding was higher than the previous studies conducted in Jimma, Ethiopia (18.8%) (21) and Brazil (2.3%) (22). The probable reason for the high prevalence of MRSA in this study might be the better sensitivity and specificity of SCC mecA gen detection compared to phenotypic determination of MRSA. A higher isolation rate of MRSA (14%-29%) has also been reported in other African countries in which HIV comorbidity is common (18). Children with HIV represent a unique group for the acquisition of antimicrobial-resistant infections due to their frequent encounters with the health care system, need for empiric antimicrobials, and immune dysfunction. Moreover, a high isolation rate of MRSA (42.8%) was reported among health workers in Jimma, which needs greater attention on the prevention and control of nosocomial infections (23). A similar study conducted in Cape Town showed that, among HIV-positive children, approximately 77% were MRSA in which the colonization was associated with a greater degree of immune suppression (24).

Among the three samples taken from the participants, the rate of MRSA detection was highest among samples collected from the perineum (59.5%). This might be due to the presence of mobile genetic element called staphylococcal cassette chromosome that encodes penicillin-binding proteins which is mostly acquired from *S. aureus* population and/or gastrointestinal micro biota (25).

The Panton–Valentine leukocidin (PVL) gene is produced from the genetic material of a bacteriophage that infects *S. aureus*, making it more virulent. In this study, 15 PVL-positive *S. aureus* strains (11.9%) were found in the carriage group (n = 126). However, a higher prevalence of PVL (38.9%) was found in *S. aureus* strains causing abscesses and arthritis (26). Nearly all CA-MRSA strains appear to have the PVL gene, which has served as a genetic marker for identifying CA-MRSA. From our findings, the majority of MRSA colonizations were PVL gene negative. Since all of the participants were ambulatory, those colonized by the MRSA could easily transmit the pathogenic bacteria to other members of the community. Most of the time, types I to III SCCmec are large elements that typically contain additional resistance genes and are characteristically isolated from hospital-acquired MRSA (HA-MRSA) strains. Conversely, CA-MRSA is associated with types IV and V, which are smaller and lack resistance genes other than mecA (27,28).

From multivariate analysis, most of the variables were not statistically significant. This may be due to the small sample size and the consequent lack of power to identify such associations, which was a limitation of this study. However, the study sites (P = 0.04) and PVL gene (P = 0.001) have a significant association with MRSA colonization. Another limitation of this study was the inability to address the lineage of the MRSA strain and lack of HIV viral load results.

Conclusions:

From this study, we found a high prevalence of pathogenic MRSA strains among HIV positive pediatric patients in the study area. The majority of MRSA types were HA-MRSA. Therefore, strict hygienic approaches by all healthcare workers in hospitals should be implemented to reduce the chance of HA-MRSA infections. Furthermore, a large-scale study that addresses the lineage of the MRSA strain is recommended.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the institutional review board of Bahir Dar University and that of the Amhara National Regional State Health Bureau. Permission to conduct the study was obtained from the hospital administrators. Written and verbal consent was obtained from the guardian of each participant. After clearing the data, numbers have been removed and participants were de-identified prior to analysis. Positive patients were linked to their physician for the sake of treatment.

Competing interest

The authors declare that we have no competing interests.

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Authors' contributions

YZ conceived the project idea, received the budget, participated in the data collection, analysis and interpretation, and wrote the initial draft of the manuscript. ZM, MT, DM, BT participated in sample collection, data analysis and interpretation of the results. All authors read and approved the final version of the manuscript.

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