International Journal of Clinical and Experimental Medical Sciences
2023; 9(4): 65-74
http://www.sciencepublishinggroup.com/j/ijcems
doi: 10.11648/j.ijcems.20230904.12
ISSN: 2469-8024 (Print); ISSN: 2469-8032 (Online)

Colonization and Associated Risk Factors of Methicillin-Resistant *Staphylococcus aureus* Among People Living with HIV at Jimma Medical Center, Southwest Ethiopia

Mekonen Adisu[1, *], Stephen Amankwah[2, 3], Rahel Tamrat[2], Yared Alemu[2], Tadele Akeba[4], Mulualem Tadesse[2, 5]

[1]Department of Medical Laboratory Sciences, Institute of Health Sciences, Wollega University, Nekemte, Ethiopia

[2]School of Medical Laboratory Sciences, Faculty of Health Sciences, Jimma University, Jimma, Ethiopia

[3]Accra Medical Centre, Accra, Ghana

[4]Department of Statistics, College of Natural Sciences, Jimma University, Jimma, Ethiopia

[5]Mycobacteriology Research Center, Jimma University, Jimma, Ethiopia

Email address:

mekonenadisu33@gmail.com (Mekonen Adisu), amankwah.ste97@gmail.com (Stephen Amankwah), rech.tam@gmail.com (Raheal Tamrat), yared.alemu6@gmail.com (Yared Alemu), tadele.diriba@ju.edu.et (Tadele Akeba), mulualemt.tadesse@gmail.com (Mulualem Tadesse)

[*]Corresponding author

To cite this article:

Mekonen Adisu, Stephen Amankwah, Rahel Tamrat, Yared Alemu, Tadele Akeba, Mulualem Tadesse. Colonization and Associated Risk Factors of Methicillin-Resistant *Staphylococcus aureus* Among People Living with HIV at Jimma Medical Center, Southwest Ethiopia. *International Journal of Clinical and Experimental Medical Sciences*. Vol. 9, No. 4, 2023, pp. 65-74. doi: 10.11648/j.ijcems.20230904.12

Received: July 5, 2023; **Accepted**: July 20, 2023; **Published**: August 5, 2023

Abstract: Nasal colonization of Methicillin-resistant *Staphylococcus aureus* (MRSA) is a potential source of spread and a prerequisite for staphylococcal infections. MRSA colonization, which causes infection in people living with HIV (PLWHIV), could lead to a longer hospital stay, increased medical costs, and prolonged antibiotic administration. This study aims to determine the nasal colonization rate, associated risk factors, and antibiotic susceptibility patterns of MRSA among PLWHIV at Jimma University Medical Center (JUMC), Southwest Ethiopia. An institution-based cross-sectional study was conducted among PLWHIV at JUMC from July to October 2021. Data on associated risk factors were collected by using a structured questionnaire and by reviewing patients' medical records. Nasal swabs were cultured on mannitol salt agar and *S. aureus* was identified by the standard bacteriological procedures. *S. aureus* isolates were subjected to antibiotic susceptibility tests by the modified Kirby- Bauer disc diffusion method and resistance to the cefoxitin (30µg) disk signified MRSA. Binary and multivariable logistic regressions were employed to identify factors associated with MRSA nasal colonization, and a *p-value* < 0.05 was taken as statistically significant. A total of 351 PLWHIV were included in our study. The overall nasal colonization rate was 17.7% (62/351) for *S. aureus* and 6.0% (21/351) for MRSA. Hospitalization in the previous six months, 27.35 (95% CI: 4.042-185.08; $p = 0.001$), a viral load greater than 1000 copies/mL, 24.44 (95% CI: 1.885-317.12; $p = 0.014$) and rural residence, 9.49 (95% CI: 1.404-64.19; $p = 0.021$), were associated with increased odds of MRSA nasal colonization. Among the total MRSA isolates, 81.2% were multidrug resistant with the highest resistance rate against Erythromycin (85.8%), followed by Sulfamethoxazole-trimethoprim (66.6%), and Clindamycin (66.6%). Only 23.7% of MRSA isolates were resistant to Chloramphenicol and Gentamicin. A relatively low rate of MRSA nasal colonization was documented among PLWHIV. The majority of the MRSA isolates were, however, multidrug-resistant, which calls for regular screening of PLWHIV during their follow-up periods for the control and prevention of MRSA.

Keywords: Nasal Colonization, PLWHIV, *S. aureus*, MRSA, Multi-Drug resistant, Jimma University Medical Center

1. Introduction

Staphylococcus aureus is a typical commensal of the body that is found within the upper respiratory tract and on the skin. Even though *S. aureus* is a commensal, it can cause skin diseases including abscesses, respiratory infections like sinusitis, and food poisoning [1]. Moreover, it is liable for causing severe infections such as endocarditis, necrotizing pneumonitis, and osteomyelitis among others [2]. Pathogenic strains regularly advance infections by delivering harmful factors like powerful toxins and the declaration of adhesion protein that ties and inactivates antibodies [3].

Colonization and infection of Methicillin-resistant *Staphylococcus aureus* (MRSA) have been perceived more in people living with HIV (PLWHIV) than in the general population [4]. Reduced host resistance among them places them at higher risk for infections, including those that carried *S. aureus* and MRSA as supplies for resulting transmission to others [5]. Additionally, a higher predominance of pathogenic MRSA strains has been archived among PLWHIV, which indicates HIV infection as a risk component for MRSA colonization [6] and subsequent infections by the same colonizing strain [7, 8].

The prevalence of MRSA among PLWHIV in Ethiopia has been reported to be 16.8% and 37.3% among pediatric patients in Northwest Ethiopia, 2.4% in Mekelle, Northern Ethiopia, and 20.8% in Arba Minch, Southern Ethiopia [9-12]. In Jimma, Southwest Ethiopia, the colonization rate of MRSA is reported high among school children, prisoners, and medical students [13, 14]. The studies, however, included a minute percentage of HIV-infected people who do not represent the general population of PLWHIV. There was a gap in MRSA nasal colonization rate, associated risk factors, and antimicrobial susceptibility patterns among PLWHIV at Jimma University Medical Center (JUMC) during their treatment follow-up and counseling. Moreover, to the best of our knowledge, no such study exists in the Southwest region of Ethiopia. Our study, therefore, sought to fill this gap and generate up-to-date information on the colonization rate, associated risk factors, and antimicrobial resistance patterns to be available at the national and local levels to guide the rational use of existing antimicrobials among PLWHIV. Hence, our study was carried out to determine the MRSA nasal colonization rate, associated risk factors, and antimicrobial susceptibility patterns among PLWHIV attending JUMC in southwest Ethiopia.

2. Materials and Methods

2.1. Study Setting

The study was conducted at JUMC, a tertiary teaching medical center in Jimma town in southwest Ethiopia, which is 352 Km from Addis Ababa, the capital city of Ethiopia. It is one of the largest medical centers in the Oromia regional state that delivers health services in areas of emergency, gynecology and obstetrics, pediatrics, child health, surgery, psychiatry, internal medicine, dermatology, ophthalmology, anesthesiology, dentistry, antiretroviral therapy (ART) clinic, clinical laboratory, and other diagnostic services. There are different units in the laboratory wing including microbiology, parasitology, hematology and immunohematology, clinical chemistry, and serology that provide laboratory services for the clients. The ART clinic at JUMC provides counseling, initiation treatment, CD4 count for newly enrolled and viral load for follow-up care. During the study period, the clinic serves 3062 PLWHIV with regular three months follow-up which includes a physical examination, counseling, medical test, routine viral load three months-, six months-, and twelve months- post ART initiation, and annual viral load test.

2.2. Study Design and Period

An institution-based cross-sectional study was conducted from July to October 2021 among PLWHIV attending the ART clinic at JUMC.

2.3. Recruitment of Study Participants

A total of 351 study participants (aged ≥ 18 years) were consecutively recruited from 3062 PLWHIV during their regular follow-up visits to the ART clinic at JMC for ART treatments, medical checkups, viral load tests and counseling sessions. Participants with nasal infections (swollen and bleeding) and/or on antibiotics during the data collection period were excluded. A single population proportion formula was used to calculate the sample size of 351 with the following assumptions; 20.8% prevalence of MRSA nasal colonization among PLWHIV from a previous study [12], 95% confidence level, 4% margin of error, and 10% non-response rate.

2.4. Data Collection Procedure

2.4.1. Socio-Demographic Characteristics and Associated Risk Factors

Data on socio-demographic characteristics and associated risk factors, such as the previous history of hospitalization, presence of a chronic wound, opportunistic infection, CD4 count, viral load, invasive procedure, history of diabetes mellitus, history of chronic diseases, AIDS clinical-stage, and history of repeated antibiotic usage were collected using structured questionnaires and from participants' medical records by trained health professionals. The questionnaire was prepared in English and translated into Afan Oromo and Amharic, as study participants either spoke one or both local languages.

2.4.2. Nasal Swab Collection

Nasal swabs were collected from each participant's anterior nares with sterile cotton swabs moistened with sterile normal saline. Briefly, a cotton swab was inserted approximately 2-3 cm into one nostril and rotated gently against the inner surface 3-5 times, and repeated to the 2nd nostril using the same cotton swab by trained health professionals. The collected nasal specimens were placed in test tubes containing Amies transport medium (Oxoid, UK), labeled with a study ID, time of collection, and transported to the Medical Microbiology Laboratory of Jimma University using a cold chain within 2 hours of collection.

2.5. Laboratory Procedure

2.5.1. Culturing and Isolation of Staphylococcus Aureus

At the microbiology laboratory, nasal specimens were cultured onto mannitol salt agar (Oxoid, UK) and incubated at 37°C for 24- 48 hours. Characteristic golden yellow or cream colonies with yellowish backgrounds rising from the overnight culture were noted as presumptive *S. aureus* colonies. The colonies were further characterized by standard bacteriological procedures such as Gram reaction, catalase test, and coagulase test. Colonies that were Gram-positive, catalase-positive, and coagulase-positive were confirmed as *S. aureus*.

2.5.2. Isolation of MRSA

Staphylococcus aureus isolates were inoculated on nutrient agar (Oxoid, UK) and the susceptibility to Cefoxitin was tested to identify MRSA [15]. All the isolates were subjected to a Cefoxitin disc diffusion test using a 30µg disc. A 0.5 McFarland standard suspension of the isolates was made and inoculated on a Mueller Hinton agar plate and then a cefoxitin disk was placed. Plates were incubated at 37°C for 18–24 hours and inhibition zone diameters (mm) were measured. An inhibition zone diameter of ≤ 21mm was reported as Methicillin/Cefoxitin resistant and ≥ 22mm was considered as Methicillin/Cefoxitin susceptible according to the guideline of the Clinical and Laboratory Standards Institute (CLSI) [15].

2.5.3. Antimicrobial Susceptibility Testing

Following the identification of MRSA, antimicrobial susceptibility testing was carried out by using Kirby–Bauer's disk diffusion method. Briefly, 0.05 McFarland suspensions of MRSA and MSSA were prepared and inoculated on Mueller-Hinton agar. Then antibiotic discs were placed on the surface of the inoculated agar as recommended by the CLSI guideline [15]. The following antibiotics (Oxoid, UK) were tested: lincosamide; clindamycin (2µg), amphenicol; chloramphenicol (30µg), macrolide; erythromycin (15µg), aminoglycoside; gentamicin (10 µg) and amikacin (30 µg), sulfonamide; sulfamethoxazole-trimethoprim (25 µg), and tetracycline; tetracycline (30 µg). The diameter of inhibition around the discs was measured to the nearest millimeter and interpreted as sensitive (S), intermediate (I), or resistant (R) according to the defined breakpoints of CLSI guidelines [15]. A control strain of *S. aureus* ATCC 25923 was used to monitor the effectiveness of antimicrobial discs and inoculating media.

2.6. Data Processing and Analysis

Data were entered into Epi data 3.1 version software, checked for inconsistencies and cleaned accordingly, and analyzed by SPSS software version 25. Descriptive analysis, such as frequencies was used. The binary and multivariable regression and adjusted odds ratios were calculated to see whether there is a statistically significant association between dependent and independent variables. All variables with a *p*-value less than 0.25 in the bivariate analysis were included in the multivariable model. Statistical significance was set at *p-value* < 0.05.

3. Results

3.1. Socio-Demographic Characteristics of the Study Participants

A total of 351 PLWHIV were included in our study. Females accounted for 61.5% (216/351) of the total. The mean age of study participants was 39.7± 9.8 years. The majority of the participants in our study were between the ages of 35 and 44, with a maximum age of 75 years. Approximately, 85% (299/351) had a residency in Jimma and the nearby towns, with the rest living in rural areas (Table 1).

Table 1. *Distribution of S. aureus and MRSA by the sociodemographic characteristic of study participants among PLWHIV at JUMC, Southwest Ethiopia, from July to October 2021.*

Variables	Categories	N (%)	*S. aureus*		
			Culture Negative	Culture Positive	
				MRSA	MSSA
Sex	Male	135 (38.5)	113 (83.7)	8 (5.9)	14 (10.4)
	Female	216 (61.5)	176 (81.5)	13 (6.0)	27 (12.5)
Age	18-24	23 (6.6)	17 (73.9)	1 (4.3)	5 (21.3)
	25-34	71 (20.2)	58 (81.7)	6 (8.4)	7 (9.8)
	35-44	141 (40.5)	116 (82.3)	8 (5.7)	17 (12.1)
	45-54	89 (25.4)	75 (84.3)	5 (5.6)	9 (10.1)
	55-64	25 (7.1)	21 (84)	1 (4)	3 (12.0)
	>=65	2 (0.6)	2 (100)	0	0
Residence	Urban	299 (85.2)	245 (81.9)	16 (5.3)	38 (12.7)
	Rural	52 (14.8)	44 (84.6)	5 (9.6)	3 (5.7)
Occupation	Government	69 (19.7)	62 (89.8)	1 (1.4)	6 (8.6)
	Private	77 (21.9)	66 (85.7)	4 (5.2)	7 (9.1)
	Merchant	48 (13.7)	41 (85.4)	4 (8.3)	3 (6.2)
	Daily laborer	75 (21.4)	58 (77.3)	7 (9.3)	10 (13.3)
	Housewife	33 (9.4)	27 (81.8)	2 (6.1)	4 (12.1)
	Cleaner	19 (5.4)	14 (73.7)	1 (5.2)	4 (21.1)
	Student	17 (4.8)	11 (64.7)	1 (5.8)	5 (29.4)
	Farmer	13 (3.7)	10 (76.9)	1 (7.6)	2 (15.4)

N= Number; MRSA= Methicillin-resistant *S. aureus*; MSSA= Methicillin-sensitive *S. aureus*

3.2. Clinical Characteristics of Study Participants

All the participants included in our study were on ART. In the previous six months, 10% (36/351) of the participants had been hospitalized. Ninety-four percent (331/351) of the participants were on a first-line regimen, 5.1% (18/351) were on a second-line regimen, and 47.3% (166/351) had been on ART for more than 10 years. Almost 5% (17/351) of individuals had previously encountered ART failure and 6% (21/351) had a history of pneumonia. Seventy-seven percent (269/351) of participants had undetectable viral loads and 95.7% (336/351) were in WHO clinical stage I. The median and range of durations on ART were 9.8 years and 21 years respectively, while the minimum duration was 3 months (Table 2).

Table 2. *Distribution of S. aureus and MRSA by clinical characteristics of study participants among PLWHIV at JUMC, Southwest Ethiopia, from July to October 2021.*

Variables	Categories	N (%)	*S. aureus*		
			Culture Negative	Culture Positive	
				MRSA	MSSA
Hospitalization in the previous six months	Yes	36 (10.3)	24 (66.7)	10 (27.8)	2 (5.6)
	No	315 (89.7)	265 (84.1)	11 (3.5)	39 (12.4)
Household members hospitalized in the past year	Yes	18 (5.1)	14 (77.8)	3 (16.7)	1 (5.5)
	No	333 (94.8)	275 (82.6)	18 (5.4)	40 (12.0)
Presence of wounds in the last past year	Yes	22 (6.3)	15 (68.2)	4 (18.2)	3 (13.6)
	No	329 (93.7)	274 (83.3)	17 (5.2)	38 (11.6)
Antibiotics in the last three months	Yes	75 (21.4)	60 (80.0)	7 (9.3)	8 (10.7)
	No	276 (78.6)	229 (82.9)	14 (5.1)	33 (11.9)
Surgery in the last year	Yes	6 (1.7)	5 (83.3)	0	1 (16.7)
	No	345 (98.3)	284 (82.3)	21 (6.1)	40 (11.6)
Presence of diabetes mellitus	Yes	7 (2.0)	5 (71.4)	0	2 (28.6)
	No	344 (98.0)	284 (82.6)	21 (6.1)	39 (11.3)
Type of ART regimen	First-line	331 (94.3)	276 (83.4)	16 (4.8)	39 (11.8)
	Second-line	18 (5.1)	11 (61.1)	5 (27.8)	2 (11.1)
	Third-line	2 (0.6)	2 (100)	0	0
Duration on ART in year	<=1	18 (5.1)	15 (83.3)	1 (5.6)	2 (11.1)
	2-5	39 (11.1)	32 (82.1)	3 (7.7)	4 (10.3)
	6-10	128 (36.5)	103 (80.5)	8 (6.3)	17 (13.3)
	>10	166 (47.3)	139 (83.7)	9 (5.4)	18 (10.8)
History of ART failure	Yes	17 (4.8)	10 (58.8)	5 (29.4)	2 (11.8)
	No	334 (95.2)	279 (83.5)	16 (4.8)	39 (11.7)
History of pneumonia	Yes	21 (6.0)	11 (52.4)	7 (33.3)	3 (14.3)
	No	330 (94.0)	278 (84.2)	14 (4.2)	38 (11.5)
History of Tuberculosis	Yes	84 (23.9)	73 (86.9)	4 (4.8)	7 (8.3)
	No	267 (76.1)	216 (80.9)	17 (6.4)	34 (12.7)
Renal diseases	Yes	7 (2.0)	4 (57.1)	2 (28.6)	1 (14.3)
	No	344 (98.0)	285 (82.8)	19 (5.5)	40 (11.6)
Liver diseases	Yes	3 (0.9)	3 (100)	0	0
	No	348 (99.1)	286 (82.3)	21 (6.0)	41 (11.8)
Heart diseases	Yes	4 (1.1)	3 (75)	0	1 (25)
	No	347 (98.9)	286 (82.4)	21 (6.1)	40 (11.5)
History of Cryptococcal meningitis	Yes	9 (2.6)	1 (11.1)	5 (55.5)	3 (33.3)
	No	342 (97.4)	288 (84.2)	16 (1.7)	41 (11.9)
WHO clinical stage	Stage I	336 (95.7)	282 (83.9)	14 (4.1)	40 (11.9)
	Stage II	9 (2.6)	4 (44.4)	4 (44.4)	1 (11.1)
	Stage III	4 (1.1)	2 (50)	2 (50)	0
	Stage IV	2 (0.6)	1 (50)	1 (50)	0
Recent viral load (copies/mL)	undetectable	269 (76.6)	231 (85.9)	9 (3.3)	29 (10.7)
	20-1000	57 (16.2)	40 (70.2)	6 (10.5)	11 (19.3)
	>1000	16 (4.6)	9 (56.2)	6 (37.5)	1 (6.2)
	Not applicable	9 (2.6)	9 (100)	0	0
Recent CD4 (cells/μL)	<200	19 (5.4)	14 (73.7)	4 (21.1)	1 (5.3)
	200-349	71 (20.2)	56 (78.9)	9 (12.7)	6 (8.5)
	350-499	64 (18.2)	58 (90.6)	2 (3.1)	4 (6.2)
	>=500	190 (54.1)	154 (81.1)	6 (3.2)	30 (15.8)

N= Number; MRSA= Methicillin-resistant *S. aureus*; MSSA= Methicillin-sensitive *S. aureus;* ART= Antiretroviral therapy; WHO= World Health Organization

3.3. Colonization Rate of S. aureus and MRSA

From the total of 351 PLWHIV enrolled in our study, 17.7% (62/351) were culture positive for *Staphylococcus aureus*. Among the positive study participants, 11.4% (40/351) were females and the remaining 6.3% (22/351) were males. *S. aureus* was most prevalent in urban residents, 15.4% (54/351), and participants aged 35 – 44, 7.1% (25/351) (Table 1). MRSA was detected in 21 (33.8 %) of the 62 *S. aureus* isolates. The remaining 41 (66.2%) were shown to be methicillin-sensitive. The overall prevalence of MRSA nasal colonization was 6.0% (21/351) in our study. MRSA was more common in female study participants, 3.7% (13/351) than in male study participants, 2.3% (8/351), people with a history of hospitalization in the previous six months, 2.8% (10/351), and participants with a viral load greater than 1000 copies/mL, 1.7% (6/351).

Our study reported high MRSA nasal colonization among participants who had CD4 less than 200 cells/μL, followed by participants who had 200-349 cells/μL, participants who had been hospitalized in the previous six months, participants within a first-line regimen, and those who had been on ART for more than six years. The median and range of duration on ART were 9.8 years and 21 years while the minimum duration was three months as indicated in Table 2 with nasal colonization rate of *S. aureus,* MSSA, and MRSA.

3.4. Factors Associated with MRSA Nasal Colonization

Socio-demographic characteristics and associated factors with MRSA were analyzed using binary logistic regression. From the binary variable logistic regression, MRSA colonization was significantly associated with residence, hospitalization in the previous six months, presence of wound in the last one year, taking an oral antibiotic in the last three months, type of regimen, history of ART failure, history of pneumonia, history of Cryptococcal meningitis, viral load and CD4, with p-value < 0.25 Table 3.

Table 3. *Bivariate and multivariate logistic regression analysis of risk factors for MRSA colonization among PLWHIV at JUMC, Southwest Ethiopia, from July to October 2021.*

Variables	Categories	MRSA	MSSA	COR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Residence	Urban	16	38	R		R	
	Rural	5	3	3.958 (.844-18.574)	0.081	9.49 (1.404-64.19)	0.021
Hospitalization in the previous six months	Yes	10	2	17.727 (3.374-93.149)	0.001	27.35 (4.042-185.08)	0.001
	No	11	39	R		R	
Household members hospitalization	Yes	3	1	0.667 (0.648-68.556)	0.111	8.711 (0.344-220.585)	0.189
	No	18	40	R		R	
Presence of wound in past one year	Yes	4	3	2.980 (0.600-14.798)	0.182	0.618 (0.025-14.97)	0.767
	No	17	38	R		R	
Oral antibiotics in the last three months	Yes	7	8	2.062 (0.626-6.790)	0.234	0.211 (0.022-1.976)	0.173
	No	14	33	R		R	
Type of regimen	First-line	16	39	0.164 (0.029-0.935)	0.042	2.274 (.011-465.15)	0.762
	Second-line	5	2	R			
History of ART failure	Yes	5	2	6.094 (1.07-34.718)	0.042	2.051 (0.147-28.63)	0.593
	No	16	39	R			
History of pneumonia	Yes	7	3	6.333 (1.435-27.957)	0.015	0.988 (0.27-36.351)	0.995
	No	14	38	R			
History of Cryptococcal meningitis	Yes	5	3	3.958 (0.844-18.574)	0.081	1.193 (0.046-30.8)	0.925
	No	16	41	R		R	
Recent viral load (copies/mL)	undetectable	9	29	R		R	
	20-1000	6	11	1.758 (.506-6.101)	0.374	2.623 (.513-13.4)	0.246
	>1000	6	1	19.33 (2.04-182.55)	0.010	24.44 (1.885-317.12)	0.014
Recent CD4 (cells/μL)	<200	4	1	20 (1.888-211.842)	0.013	1.496 (0.043-52.081)	0.824
	200-349	9	6	7.5 (1.935-29.069)	0.004	4.201 (0.554-31.863)	0.165
	350-499	2	4	2.5 (0.370-16.888)	0.347	4.434 (0.403-48.768)	0.225
	>=500	6	30	R		R	

Key: R= reference category; COR= Crude odd ratio; AOR= Adjusted odd ratio; CI= Confidence Interval; MRSA= Methicillin-resistant *Staphylococcus aureus*; MSSA= Methicillin-sensitive *Staphylococcus aureus.*

All variables with p < 0.25 in the bivariate analysis were included in a multivariable model. However, from the multivariable logistic regression, only residence, history of hospitalization in the previous six months, and viral load greater than 1000 copies/mL were significantly associated with MRSA nasal colonization with a p-value < 0.05 as shown in Table 3. Study participants with a history of hospitalization in the previous six months had 27.35 (95% CI: 4.042-185.08; p = 0.001) times the risk of being MRSA nasal colonized as compared to those without a history of hospitalization. Likewise, participants with greater than 1000 viral load copies/mL had 24.44 (95% CI: 1.885-317.12; p = 0.014) times the risk of being MRSA colonized compared to participants with undetectable viral load copies. Participants who lived in rural areas had 9.49 (95% CI: 1.404-64.19; p = 0.021) times the risk of being MRSA colonized compared to participants who lived in Jimma and nearby towns.

3.5. Antimicrobial Susceptibility Pattern of S. aureus

A total of 62 *S. aureus* isolates were subjected to

antimicrobial susceptibility tests against selected antimicrobial agents. *S. aureus* isolates were 88% susceptible to chloramphenicol followed by gentamicin (87%), amikacin (74.2%), and cefoxitin (66%). However, *S. aureus* isolates were 14.5%, and 37% susceptible to erythromycin and sulfamethoxazole-trimethoprim, respectively (Table 4).

Table 4. *Susceptibility pattern of Staphylococcus aureus isolates among PLWHIV, at JUMC, southwest Ethiopia from July to October 2021.*

Antibiotic agents	*S. aureus*		
	Susceptible	Intermediate	Resistant
Cefoxitin	41 (66.2%)	--	21 (33.8%)
Erythromycin	9 (14.5%)	22 (35.5%)	31 (50%)
Clindamycin	32 (51.6%)	26 (41.9)	4 (6.5%)
Sulfamethoxazole-trimethoprim	23 (37.1%)	11 (17.7%)	28 (45.2%)
Tetracycline	33 (53.2%)	2 (3.2%)	27 (43.6%)
Chloramphenicol	55 (88.7%)	3 (4.8%)	4 (6.5%)
Gentamicin	54 (87%)	4 (6.5%)	4 (6.5%)
Amikacin	46 (74.2%)	14 (22.3%)	2 (3.5%)

3.6. Antimicrobial Susceptibility Pattern of MRSA

The susceptibility of MRSA to antimicrobial agents that are commonly used to treat infections caused by MRSA was performed. Among 21 MRSA isolates, high resistance was exhibited against erythromycin (85.8%) followed by clindamycin and sulfamethoxazole-trimethoprim (66.6%), and Tetracycline (47.2%). Chloramphenicol and gentamicin were the most effective drugs against MRSA with (76.3%) susceptibility followed by amikacin (66.7%). The detail is presented in Table 5.

Table 5. *Susceptibility Patterns of MRSA isolates among PLWHIV at JUMC, Southwest Ethiopia, July to October 2021.*

Antibiotic agents	MRSA		
	Resistant	Intermediate	Susceptible
Erythromycin	9 (42.9%)	9 (42.9%)	3 (14.2%)
Clindamycin	1 (4.7%)	13 (61.9%)	7 (33.4%)
Sulfamethoxazole-trimethoprim	10 (47.6%)	4 (19.0%)	7 (33.4%)
Tetracycline	9 (42.9%)	1 (4.7%)	11 (52.4%)
Chloramphenicol	3 (14.2%)	2 (9.5%)	16 (76.3%)
Gentamicin	3 (14.2%)	2 (9.5%)	16 (76.3%)
Amikacin	1 (4.7%)	6 (28.6%)	14 (66.7%)

3.7. Multidrug Resistance Pattern of MRSA

Multidrug resistance in this study was taken as resistance to at least one agent in three or more of the antimicrobial classes tested. The multidrug resistance status of MRSA isolates was tested against six classes of antimicrobial agents. Among the total MRSA isolates; 17/21 (81.2%) isolates were multidrug resistant. Of them, 7/21 (33.4%) showed resistance to three different classes of antimicrobials, 5/21 (23.9%) isolates showed resistance to four antimicrobial agents and one isolate was fully resistant to all tested antimicrobial agents. The details are presented in Table 6.

Table 6. *Distribution of multidrug resistance of MRSA isolates among PLWHIV at JUMC, Southwest Ethiopia, from July to October 2021.*

Number of drugs	Antibiotic agents	Frequency of resistant isolates
Resistant to 3 drugs	E, DA, GEN	1 (4.8%)
	E, DA, CHL	2 (9.5%)
	E, DA, SXT	2 (9.5%)
	E, SXT, TET	1 (4.8%)
	E, DA, TET	1 (4.8%)
	Total	7 (33.4%)
Resistant to 4 drugs	E, TET, SXT, AMI	1 (4.8%)
	E, SXT, TET, AMI	2 (9.5%)
	E, DA, SXT, AMI	1 (4.8%)
	E, SXT, TET, GEN	1 (4.8%)
	Total	5 (23.9%)
Resistant to 5 drugs	E, DA, SXT, CHL, GEN	1 (4.8%)
	E, DA, SXT, TET, AMI	1 (4.8%)
	E, SXT, TET, CHL, AMI	1 (4.8%)
	Total	3 (14.4%)
Resistant to 6 drugs	E, DA, SXT, TET, GEN, AMI	1 (4.8%)
	E, DA, SXT, TET, CHL, GEN	1 (4.8%)
	Total	2 (9.5%)

N.B: E= Erythromycin, DA= Clindamycin, SXT= Sulfamethoxazole-trimethoprim, TET= Tetracycline, CHL= Chloramphenicol, GEN= Gentamicin, AMI= Amikacin

4. Discussion

In this study, the overall nasal colonization of *S. aureus* and MRSA among people living with HIV (PLWHIV) was 17.7% and 6.0%, respectively. The presence of such a colonization rate of MRSA isolates in the nasal cavity of PLWHIV alerts us about the potential for MRSA to spread and cause serious infections in PLWHIV. A study by Netanya Utay and colleagues on PLWHIV with MRSA infection showed insufficient immune responses which results in a reduction in the number of cells expressing the cytokine IFNγ and a reduction in the function of IFNγ-producing cells. Reduced production of upstream drivers of IFNγ production, such as IL-12 and IL-15, could be the cause of the drop in IFNγ-producing cells. This antigen-specific deficiency could have far-reaching consequences because IFNγ promotes T-cell proliferation and antigen response, as well as macrophage and neutrophil responses [16, 17].

The carriage rate of MRSA in this study is comparable with studies done in Singapore, 5.1% [18], Ghana, 5.6% [19], and Uganda, 5.02% [20] among PLWHIV. Moreover, a recent meta-analysis conducted on the combined prevalence of MRSA among PLWHIV in different regions of the world was found to be 6.9%, [21] which is in line with our study findings. However, it is lower than studies done in the USA 13% [22], India, 36.11% [23], Baltimore, 15.4% [24], Iran, 12.8% [25], and Arba Minch, Southern Ethiopia, 20.8% [12]. The study finding is higher than similar studies in Brazil, 2.4% [26], Taiwan, 3.4% [27], Spain, 1% [22], Botswana, 3.2% [28], and Mekelle, northern Ethiopia, 2.4 % [11]. The relatively low *S. aureus* carriage rate reported in our study could be due to the direct inoculation of the nasal swabs on Mannitol salt agar without enrichment which may reduce the recovery rate.

Potential factors were studied to check if they had any associations with MRSA nasal colonization. Concerning residency, *S. aureus* carriage was lower among rural residents compared to urban residents. Nonetheless, MRSA colonization was higher among rural residents, with a significant association (p-value of 0.02). This result is in line with a study done in Iraq among urban and rural school communities and could be attributed to a lack of personal hygiene among rural participants [29]. Our findings, thus, highlight the need for infection prevention methods, such as the use of disinfectants, frequent hand washing, and proper sanitary practices among rural residents.

Regarding the history of hospitalization in the previous six months, study participants with a history of hospitalization had more colonization rate. This result is significantly associated with a p-value of 0.001 and is in line with other studies done in Mekelle, Ethiopia [11], and Taiwan [30]. This could be attributed to staying in hospital are most at risk to get MRSA, as they often have a way for the bacteria to get into their body, such as a wound, burn, feeding tube, drip into a vein or urinary catheter and they may have other serious health problems since their body is less able to fight off the *S. aureus*. In addition, antibiotics are routinely administered, overused, and consumed in hospital settings, potentially leading to the development of drug-resistant superbugs such as MRSA.

Regarding the duration of ART, the colonization rate was high among participants who had had more than two years of ART duration, though not statistically significant. This could be due to, repeated antibiotic usage or ART influencing the composition of the gut microbiota, especially integrase strand transfer inhibitors or non-nucleotide reverse transcriptase inhibitor-containing regimens, which might increase the colonization of MRSA in people living with HIV under long-term ART [31].

The colonization of MRSA among the study participants with a previous history of pneumonia was higher than those without previous history of pneumonia but it is not significantly associated ($p > 0.05$). This could be because MRSA colonization can lead to MRSA pneumonia or other infections in compromised hosts. MRSA pneumonia in HIV-infected people could be health-care-associated, related to bacteremia, or community-acquired, as it is in the general population. MRSA is a prevalent cause of nosocomial pneumonia in HIV-infected people (25% of cases are caused by *S. aureus*, 65% of which are methicillin-resistant), and it has been established as an independent risk factor for mortality in HIV-infected patients [3].

HIV viral load and CD4 counts are the two surrogate markers of ART responses and HIV/AIDS disease progression. In our study, high colonization of MRSA was reported among participants with CD4 <200 cells/μL followed by 200-349 cells/μL. This could be attributed to the decreased level of immunity, although it is not significantly associated ($p > 0.05$), this finding is consistent with a previous study suggesting that the colonization of MRSA in PLWHIV might be independent of CD4 T-lymphocyte counts [32]. On the other hand, high colonization of MRSA was reported among participants who had greater than 1000 copies/mL of viral load followed by participants with a viral load of 20 to 1,000 copies/mL and undetectable. This could be because when viral copies proliferate, the overproduction of inflammatory cytokines may enable secondary bacterial infections and enhance host immunopathology.

In the present study, various levels of antibiotic resistance were documented among MRSA isolates. The MRSA isolates showed a higher rate of resistance to erythromycin, sulfamethoxazole-trimethoprim, clindamycin, and tetracycline, which are consistent with earlier studies conducted in Ethiopia's northern and northwest regions [10, 11]. The increased resistance of MRSA isolates to these antibiotics, which are commonly used to treat MRSA infections, could be due to continuous genetic variation (*mecA*) of the strains caused by mutation of an existing gene or horizontal transfer of a resistance gene from another bacterium and alteration of PBP2A. Resistance to sulfamethoxazole-trimethoprim is very common among MRSA isolates and could be because sulfamethoxazole-trimethoprim is commonly used as a

prophylactic for opportunistic infections in PLWHIV.

Multidrug resistance to MRSA is currently considered a global threat by WHO [33]. The results of this study revealed the emergence of high multidrug-resistant MRSA among PLWHIV. Among the total MRSA isolates, 81.2% were multidrug-resistant. Misuse and overuse of antibiotics in the study area, poor infection prevention practices or the emergence of drug resistance strains could account for the high rate of multidrug-resistant MRSA. Furthermore, in resource-poor countries like Ethiopia, antibiotics are readily available without prescription and are dispensed indiscriminately without routine culture and antimicrobial tests, leading to an increase in antimicrobial resistance. Health information on the appropriate use of antibiotics and infection prevention practices should be delivered to healthcare practitioners, dispensers, and patients to mitigate ever-increasing antimicrobial resistance.

In our study, there are some antibiotics (such as gentamicin, chloramphenicol, and amikacin) which remain effective against MRSA. Two studies from Ethiopia (Arba Minch and Mekelle), reported similar patterns of susceptibilities for gentamicin, chloramphenicol, and amikacin [11, 12]. Moreover, gentamicin was found to be the most effective drug against *S. aureus*. One reason for its success may be that gentamicin is infrequently used and is administered by injection. Self-medication is far more difficult with this method of administration than with antibiotics prescribed orally [34]. Thus, where it is available, it is worth performing routine culture and antimicrobial susceptibility testing for selecting the most effective antibiotics against MRSA. This will help to improve proper management and reduces complications, poor outcomes and death among PLWHIV.

Our study did not determine MRSA strains involved in nasal colonization of People living with HIV as we did not employ molecular techniques to identify the strain as hospital or community acquired.

5. Conclusion

MRSA isolates found in the nasal cavity indicate a risk of MRSA spreading and subsequent infections among PLWHIV. Rural residence, history of hospitalization in the previous six months, and viral load greater than 1000 copies/mL were significantly associated with MRSA nasal colonization. The isolated MRSA, on the other hand, had the highest antibiotic resistance, and the majority of the isolates were multidrug-resistant which indicates the colonization is more caused by hospital-acquired strains. These data imply that the performance of antimicrobial therapy in infection control efforts could significantly affect the occurrence of MRSA in the hospital context. Furthermore, MRSA control and prevention are greatly aided by screening PLWHIV during their follow-up.

List of Abbreviations

ART: Antiretroviral Therapy
CLSI: Clinical and Laboratory Standards Institute
JUMC: Jimma University Medical Center
MRSA: *Methicillin-Resistant Staphylococcus aureus*
PLWHIV: People Living With HIV

Ethics Approval and Informed Consent

The study was conducted after obtaining ethical clearance and approval from the Institutional Review Board of the Institute of Health of Jimma University with reference number (JHRPGN/167/2021). Official permission was sought from JUMC. Similarly, after a clear discussion about the actual study or explaining the purpose of the study, written informed consent was obtained from each study participant. Confidentiality of the test results of the participants was maintained and the test result was used only for research purposes. Study participants with MRSA isolates were made known of their results and were connected to clinicians.

Data Availability

The data sets that support the findings of our study are available from the corresponding authors upon reasonable request.

Author Contributions

Mekonen Adisu: conceived and designed the study, experimented with and prepared the first drafted manuscript. Stephen Amankwah: Collected clinical data, assisted with laboratory work and edited the first draft of the manuscript. Mulualem Tadesse and Yared Alemu: Verified the methods, assisted data analysis and interpretation, critically revised the manuscript and jointly supervised the study. Rahel Tamirat: assisted the laboratory work, and participated in data analysis and interpretation. Tadele Akeba: analyzed and interpreted the data, and carefully reviewed the manuscript. All authors discussed the results, and read and approved the final version of the manuscript.

Competing Interests

We authors declare that we have no competing interests.

Funding

This study was financially supported by the Institute of Health of Jimma University, Ethiopia.

Acknowledgements

We are very grateful to the study participants for their kind cooperation during data collection. We would also like to express our sincere thanks to the Jimma University Medical Microbiology Laboratory for its assistance and guidance during laboratory work. Moreover, our appreciation goes to the management and staff of

the ART clinic of Jimma University Medical Center for permitting us to conduct this study.

References

[1] Sataloff RT, Johns MM, Kost KM. Staphylococcus aureus. First Edit. Fetsch A, editor. United Kingdom: Academic Press; 2018. 13–141 p. Available from: www.elsevier.com/permissions.

[2] Turner NA, Sharma-kuinkel BK, Maskarinec SA, Emily M, Shah PP, Carugati M, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol. 2020; 17 (4): 203–18. Available from: doi: 10.1038/s41579-018-0147-4.

[3] Hidron A, Moanna, M. D. A, Russell Kempker MD, David Rimland MD. Methicillin-resistant Staphylococcus aureus in HIV-infected patients. Infection and Drug Resistance. 2010; 3 (5): 73–86. Available from: http//dx.doi.10.2147/idr.s7641

[4] Shet A, Mathema B, Mediavilla JR, Kishii K, Mehandru S, Jeane-Pierre P, et al. Colonization and subsequent skin and soft tissue infection due to methicillin-resistant Staphylococcus aureus in a cohort of otherwise healthy adults infected with HIV type 1. Journal of Infectious Diseases. 2009; 200 (1): 88–93. Available from: doi: 10.1086/599315.

[5] Panigrahy A, Sinha S, Das BK, Kapil A, Vishnubhatla S, Dhawan B. Staphylococcus aureus Colonisation in HIV-Infected Patients : Incidence, Risk Factors and Subsequent Skin-and Soft-Tissue Infections. Indian Journal of Medical Microbiology. 2020; 38 (3–4): 444–7. Available from: https://doi.org/10.4103/ijmm.IJMM_20_5

[6] Zervou FN, Zacharioudakis IM, Ziakas PD, Rich JD, Mylonakis E. Prevalence of and risk factors for methicillin-resistant staphylococcus aureus colonization in HIV infection: A meta-analysis. Clinical Infectious Diseases. 2014; 59 (9): 1302–11. Available from: doi: 10.1093/cid/ciu559.

[7] Ragavan Rameshkumar M, Arunagirinathan N. Drug-Resistant Bacterial Infections in HIV Patients. Advances in HIV and AIDS Control. Chennai, India; 2018. 84–102 p. Available from: http://dx.doi.org/10.5772/intechopen.78657

[8] Shahcheraghi S, Ayatollahi J, Niri M, Fazilati A. The most common bacterial infections in HIV-infected patients. Medical Journal of Dr DY Patil University. 2016; 9 (6): 773.

[9] Zenebe Y, Tibebu M, Tulu B, Mekonnen D, Mekonnen Z. Methicillin-resistant Staphylococcus aureus with genotyping method among human immunodeficiency virus positive pediatric patients in Northwest Ethiopia: A cross-sectional study design. Ethiopian Journal of Health Development. 2018; 32 (3): 1–8.

[10] Lemma MT, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z. Methicillin resistant staphylococcus aureus among HIV infected pediatric patients in northwest Ethiopia: Carriage rates and antibiotic co-resistance profiles. PLoS ONE. 2015; 10 (9): 1–10. Available from: doi: 10.1371/journal.pone.0137254.

[11] Gebremedhn G, Gebremariam TT, Wasihun AG, Dejene TA, Saravanan M. Prevalence and risk factors of methicillin-resistant Staphylococcus aureus colonization among HIV patients in Mekelle, Northern Ethiopia. SpringerPlus. 2016; 5 (877): 1–9. Available from: DOI 10.1186/s40064-016-2613-7.

[12] Manilal A, Shewangizaw M, Mama M, Gezmu T, Merdekios B. Methicillin-resistant Staphylococcus aureus colonization in HIV patients of Arba Minch Province, Ethiopia: Carriage rates, antibiotic resistance, and biofilm formation. Acta Microbiologica et Immunologica Hungarica. 2019; 66 (4): 469–83. Available from: doi: 10.1556/030.66.2019.014.

[13] Efa F, Alemu Y, Beyene G, Gudina EK, Kebede W. Methicillin-resistant Staphylococcus aureus carriage among medical students of Jimma University, Southwest Ethiopia. Heliyon. 2019; 5 (1): e01191. Available from: https://doi.org/10.1016/j.heliyon.2019.e01191

[14] Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant Staphylococcus aureus (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. Annals of Clinical Microbiology and Antimicrobials. 2013; 12 (11): 1–11. Available from: doi: 10.1186/1476-0711-12-11 · Source: PubMed.

[15] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. Clinical and Laboratory Standards Institute; 2021. M100 CLSI supplement. 2021; 41 (3): 1–315.

[16] Utay NS, Roque A, Timmer JK, Morcock DR, DeLeage C, Somasunderam A, et al. MRSA Infections in HIV-Infected People Are Associated with Decreased MRSA-Specific Th1 Immunity. PLoS Pathogens. 2016; 12 (4): 1–15.

[17] Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. Staphylococcus aureus nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Frontiers in Microbiology. 2018; 9 (OCT): 1–15.

[18] Kyaw WM, Lee LK, Siong WC, Ping ACL, Ang B, Leo YS. Prevalence of and risk factors for MRSA colonization in HIV-positive outpatients in Singapore. AIDS Research and Therapy. 2012; 9 (33): 1–6. Available from: http://www.aidsrestherapy.com/content/9/1/33

[19] Donkor ES, Kotey FCN, Dayie NTKD, Duodu S, Tetteh-Quarcoo PB, Osei M-M, et al. Colonization of HIV-Infected Children with Methicillin-Resistant Staphylococcus aureus. Pathogens. 2019; 8 (35): 1–14. Available from: www.mdpi.com/journal/pathogens

[20] Taremwa Ivan Mugisha, Sarah Mwebaze, Christine Atuhairwe BS. Perspective for Methicillin-resistant Staphylococcus Aureus colonization, Antibiotic Susceptibility Patterns and Risk factors for Colonization among People Living with HIV at Nyenga Hospital, Buikwe District, in Central Uganda. INTERNATIONAL JOURNAL OF INFECTION PREVENTION. 2018; 1 (1): 1–8. Available from: doi: 10.14302/issn.2690-4837.ijip-18-2238

[21] Sabbagh P, Riahi SM, Gamble HR, Rostami A. The global and regional prevalence, burden, and risk factors for methicillin-resistant Staphylococcus aureus colonization in HIV-infected people: A systematic review and meta-analysis. American Journal of Infection Control. 2019; 47 (3): 323–33. Available from: https://doi.org/10.1016/j.ajic.2018.06.023

[22] Imaz A, Camoez M, Di Yacovo S, Gasch O, Dominguez MA, Vila A, et al. Prevalence of methicillin-resistant Staphylococcus aureus colonization in HIV-infected patients in Barcelona, Spain: A cross-sectional study. BMC Infectious Diseases. 2015; 15 (1): 1–5. Available from: http://dx.doi.org/10.1186/s12879-015-0991-z

[23] Kumar S, Laskar S, Bandopadhyay M, Banerjee P. Nasal methicillin-resistant Staphylococcus aureus colonization in HIV-infected patients from eastern India. Saudi Journal for Health Sciences. 2013; 2 (1): 14. Available from: doi: 10.4103/2278-0521.112625.

[24] Farley JE, Hayat MJ, Sacamano PL, Ross T, Carroll K. Prevalence and risk factors for methicillin-resistant Staphylococcus aureus in an HIV-positive cohort. American Journal of Infection Control. 2015; 43 (4): 329–35. Available from: http://dx.doi.org/10.1016/j.ajic.2014.12.024

[25] Hassanzadeh P, Hassanzadeh Y, Mardaneh J, Rezai E, Motamedifar M. Isolation of methicillin-resistant staphylococcus aureus (MRSA) from hiv patients referring to HIV referral center, Shiraz, Iran, 2011-2012. Iranian Journal of Medical Sciences. 2015; 40 (6): 526–30.

[26] Lastoria LC, Cunha M de LR de S da, Souza CSM de, Souza L do R de, Victória C, Fortaleza CMCB. Carriage of Methicillin-resistant Staphylococcus aureus among people living with HIV-AIDS in inner São Paulo State, Brazil: molecular and spatial epidemiology. General Microbiology Immunology. 2020; 1: 1–32. Available from: https://orcid.org/0000-0003-4120-1258

[27] Hsu Y, Wu D, Hung C, Huang S, Yuan F, Lee M. Methicillin-resistant Staphylococcus aureus nasal colonization among HIV-infected patients in Taiwan : prevalence, molecular characteristics and associated factors with nasal carriage. BMC Infectious Diseases. 2020; 20 (254): 1–8. Available from: https://doi.org/10.1186/s12879-020-04979-8

[28] Reid MJA, Steenhoff AP, Mannathoko N, Muthoga C, McHugh E, Brown EL, et al. Staphylococcus aureus nasal colonization among HIV-infected adults in Botswana: prevalence and risk factors. AIDS Care - Psychological and Socio-Medical Aspects of AIDS/HIV. 2017; 29 (8): 961–5. Available from: http://dx.doi.org/10.1080/09540121.2017.1282600

[29] Hussein NR, Basharat Z, Muhammed AH, Al-Dabbagh SA. Comparative evaluation of MRSA nasal colonization epidemiology in the Urban and Rural Secondary School Community of Kurdistan, Iraq. PLoS ONE. 2015; 10 (5): 1–9.

[30] Wua CJ, Ko WC, Ho MW, Lin HH, Yange YL, Lin JN, et al. Prevalence of and risk factors for methicillinresistant staphylococcus aureus colonization among human immunodeficient virus-infected outpatients in taiwan: Oral candida colonization as a comparator. Journal of Oral Microbiology. 2017; 9 (1): 1–10. Available from: https://doi.org/10.1080/20002297.2017.1322446

[31] Imahashi M, Ode H, Kobayashi A, Nemoto M, Matsuda M, Hashiba C, et al. Impact of long-term antiretroviral therapy on gut and oral microbiotas in HIV-1-infected patients. Scientific Reports. 2021; 11 (1): 1–10. Available from: https://doi.org/10.1038/s41598-020-80247-8

[32] Abiye P, Godwin E. Nasal Carriage Prevalence of MRSA in People Living with HIV / AIDS Undertaking Antiretroviral Therapy in a Tertiary Hospital in Port Harcourt. International Journal of Innovative Healthcare Research. 2018; 6 (1): 12–23. Available from: www.seahipaj.org.

[33] World Health Organization (WHO). Antimicrobial resistance. Global Challenges and Future Interventions. GENEVA: WHO; 2017.

[34] Grecu AM, Dave DM, Saffer H. Mandatory Access Prescription Drug Monitoring Programs and Prescription Drug Abuse. Journal of Policy Analysis and Management. 2019; 38 (1): 181–209. Available from: doi: 10.1002/pam.22098.