Methicillin and Vancomycin Resistant *Staphylococcus aureus* and Associated Factors from Surgical Ward Inpatients at Debre Markos Referral Hospital, Northwest Ethiopia

Samrawit Tefera Demsie (aresieadusam@gmail.com)
Bahir Dar University https://orcid.org/0000-0002-5345-6242
Tewachew Awoke
Bahir Dar University
Daniel Mekonnen
Bahir Dar University

Research

Keywords: Wound, MRSA, VRSA, Debre Markos Referral Hospital, Ethiopia

Posted Date: December 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-133599/v1

Abstract

Background: *Staphylococcus aureus* is one of the leading wound infections. It is considered as a super bug. The epidemiology of Methicillin Resistant *S. aureus* (MRSA) and Vancomycin Resistant *S. aureus* (VRSA) is not well studied in Ethiopia particularly in Debre Markos Referral Hospital (DMRH). The objective of the study was to determine the prevalence of MRSA and VRSA and associated factors from wound cases admitted to surgical ward in DMRH, Northwest Ethiopia.

Methods: A cross-sectional study was conducted from February-April 2020 at DMRH. A structured questionnaire was used to collect demographic and clinical characteristics. Wound swabs were collected using sterile cotton swab followed by culturing on Blood agar and Mannitol Salt agar. Then isolates were characterized by gram stain and biochemical tests. The presence of MRSA and VRSA was determined using the cefoxitin (30μg) antibiotic disc diffusion and vancomycin E-test, respectively. The data was analyzed using Statistical Package for Social Sciences, version 20 software. *P*-value <0.05 was considered statistically significant.

Results: A total of 242 wound cases were enrolled and a majority of them were males (172, 71.1%). Among the total wound cases, the isolation rate of *S. aureus* was 29.3%. The proportion of MRSA was 13.22% and that of VRSA was 4.1%. The proportion of vancomycin intermediate *S. aureus* (VISA) was gauged at 4.5%. Hospital stay >72h (p=0.014), wound depth (p=0.043), antibiotic use (p=0.017) and previous history of wound infection (p=0.001) showed statistical significant association with MRSA. No variables showed significant association with VRSA.

Conclusion: High proportions of *S. aureus* isolates are resistant to methicillin and vancomycin. Moreover, multiple variables demonstrated associations with drug resistance. Hence, hospital infection control and antibiotic stewardship program shall be strengthened.

Background

Staphylococcus aureus is a leading human pathogen that causes a broad spectrum of clinical conditions. It is a leading cause of bacteremia, infective endocarditis, osteoarticular, skin and soft tissue infections (SSTI), pleuropulmonary, and device-related infections[1]. The infections might originate from health facilities (nosocomial infections) or from community (community-acquired infections) [2].

Methicillin Resistant *Staphylococcus aureus* (MRSA) is *S. aureus* that is resistant to methicillin, oxacillin, cloxacillin, dicloxacillin and nafticillin. Methicillin resistance in Staphylococci is due to the incorporation of extra chromosomal gene from plasmid; staphylococcal cassette chromosome mec (SCCmec). All SCCmec types contained the mecA gene, which codes for the low-affinity penicillin binding protein PBP2a [3]. MRSA is the predominant *S .aureus* isolates from infected wounds [1].

The current treatment options for MRSA infections include Trimethoprim, Sulfamethoxazole, Doxycycline or Minocycline, Clindamycin, and Linezolid. However, most MRSA strains are also evolutionary competent

and resistant to other class of antibiotics which are mentioned above. *S. aureus* isolated from wound infection of hospitalized patients have high degree of biofilm-forming capacity [3].

Vancomycin became an antibiotic of choice for treatment of MRSA infections in hospital settings since the late 1980s [4]. *S. aureus* strains moderately resistant vancomycin had first emerged from Japan in the year 1997, followed by the first report of vancomycin-resistant *S. aureus* (VRSA) (MIC, > 32 μg per milliliter) from the USA in the year 2002[5].

S. aureus acquire to vancomycin resistance from Vancomycin Resistant *Enterococcus* (VRE) plasmid through transposon Tn1546 [6]. Linezolid, tigecycline, daptomycin, quinupristin, dalfopristin, ceftobiprole, iclaprim, and novel glycopeptides like the dalbavancin, telavancin, and oritavancin are a few choices of antibiotics to treat infections caused by VRSA isolates [7].

Then prevalence of VRSA lied on a wide range 9.8%-52.4% globally [8, 9, 10, 11].A systematic review and meta-analysis summarized the prevalence of MRSA and VRSA among *S. aureus* isolates in Ethiopia and the prevalence showed a wide range 8.3%-77.3% (with pooled prevalence of 32.5% for MRSA [12] and 5.1% − 44.3% [7] for VRSA.

There was previous report on the prevalence of MRSA in DMRH [13]. However, this study was distant past (8 years ago) and restricted to surgical sites. The current epidemiology is unknown. Hence, the objective of the study was to determine the prevalence of MRSA and VRSA and associated factors from wound cases admitted to surgical ward in DMRH, Northwest Ethiopia.

Methods

Study setting and context

A cross sectional study was conducted among 242 study participants with wound cases at DMRH, Debre Markos, Ethiopia from February-April 2020. The hospital provides compressive services such as inpatient and outpatient treatment for people comes from the surrounding zones and nearby regional states.

Specimen collection and processing

Wound swab was collected using cotton toped sterile applicator stick. Then, the sample was inoculated on Blood agar plate and Mannitol Salt agar, incubated at 35–37 °C for 24 h. Growth was identified using biochemical tests and Gram Stain. *S. aureus* isolates were further processed for determination of their susceptibility profile.

Antimicrobial susceptibility testing

After growth, 0.5 McFarland suspensions of *S. aureus* isolates were inoculated on Mueller Hinton agar (MHA) for modified Kirby-Bauer disk-diffusion sensitivity analysis. MRSA was deciphered via 30 μg cefoxitin disk diffusion test. After incubation at 35 °Cfor 24 h, results were interpreted using CLSI guidelines[14]. All MRSA isolates were taken and inoculated on MHA to look for VRSA. A vancomycin

strip of E-test was gripped with a pair of sterile forceps, and placed on to the inoculated agar surface. After incubation at 35–37 °C for 16 to 18 h results were read and interpreted as minimum inhibitory concentration (MIC) ≤ 2 µg /ml sensitive, 2–4 ≥ intermediate and ≥ 16 µg /ml resistance to vancomycin drug based on CLSI guidelines [14].

Statistical analysis and quality assurance

Each wound swab sample was processed based on recommended standard laboratory procedures by strictly following pre-analytical, analytical and Post-analytical stages of quality assurance that are incorporated in standard operating procedures (SOPs) of the microbiology laboratory unit. Culture media was prepared aseptically by autoclaving and 5% of batch prepared media was checked after overnight incubation as a control. Additionally, the Blood agar media and Mannitol Salt agar was checked for growth of known *S. aureus* ATCC25923. *S. aureus* A TCC25923 (cefoxitin zone 23–29 mm) and *S. aureus* ATCC 43300(zone ≤ 21 mm) were used as a control strains to cefoxitin disk diffusion test for MRSA. *S. aureus* ATCC 29213 MIC value 0.5-2.0 µg/ml was used as a control strains to vancomycin MIC screening[12].

Data was cleaned, double entered and analyzed using Statistical Package for Social Science, version20 software. Initially, unilabiate logistic regression was carried out and variables with a p-value of less than 0.2 entered in to multiple logistic regression analysis, P-value of < 0.05 was considered as statistically significant.

Results

Socio-demographic and clinical characteristics of study participants

A total of 242 study participants were enrolled. Of which, the majority 172(71.1%) were males and 70 (28.9%) were females with a sex ratio of 1:0.41. The ages of study subjects ranged from 1–84 years with a mean age of 33 years and median age of 30 years. Most 93 (38.4%) of study participants were in the age group of 15–30 years and 171(70.7%) were rural dwellers. Majority 139(57.4%) sample was collected from study participants who developed wound cases after trauma (Table 1).

Table 1
Sociodemographic and clinical characteristics frequency in patients with wound cases at DMRH, February to April 2020 (n = 242)

Variables	Characteristics	N, (%) n
Sex	Male	172 (71.1)
	Female	70 (28.9)
Age (years)	< 15 years	34 (14.0)
	15–30 years	93 (38.4)
	31–45 years	64 (26.4)
	46–60 years	33 (13.6)
	> 60 years	18 (7.4)
Residence	Rural	171 (70.7)
	Urban	71 (29.3)
Type of wound	Surgical wound	46 (19.0)
	Non healing ulcer	19 (7.9)
	Burn wound	16 (6.6)
	Trauma	139 (57.4)
	Abscess	16 (6.6)
	Others	6 (2.5)
Total		242(100)

Proportion of MRSA and VRSA

Staphylococcus aureus was isolated from 242 inpatients. Cefoxitin disc diffusion was carried out to 71 *S. aureus* isolates; the proportion of MRSA among surgical ward inpatients at DMRH was 32/71 (45.1%). Similarly, the proportion of VRSA was 10/71 (14.08%; MIC ≥ 16 µg/ml) and the proportion of Vancomycin intermediate *Staphylococcus aureus* (VISA) was 11/71 (15. 5%; MIC 4–8 µg/ml). Zooming in, the proportion of VRSA among MRSA isolates was gauged at 10/32(31.25%) (Table 2).

Table 2
Antibiotic resistance pattern of *S. aureus* isolates among patients with wound cases at DMRH February to April 2020 (n = 71)

Antibiotics	inhibition		N (%),	Interpretation	Strain
Cefoxitin	I-Z	≥ 22 mm	39(54.9)	S	MSSA
		≤ 21 mm	32(45.1)	R	MRSA
Vancomycin	MIC	≤ 2 µg/ml	11(15.5%)	S	VSSA
		4–8 µg/ml	11(15.5%)	I	VISA
		≥ 16 µg/ml	10 (14.1)	R	VRSA

Note: S = Susceptible, R = Resistant, I = Intermediate, I-Z = Inhibition zone and MIC = Minimum inhibition concentration.

As shown in Table 3 equal number 16(6.6%) of MRSA isolates, were recovered from male and female participants. Majority of MRSA isolate 14 (5.9%) were in the age groups of 15–30 years and high prevalence of VRSA isolates were in the age groups of 31–45 years 6(2.48%). Eighteen (7.44%) of MRSA and 7(2.9%) VRSA isolated were from trauma wound type. MRSA and VRSA were more frequently isolated among inpatients with deep wound than superficial wound.

Table 3
Distribution of MRSA and VRSA among patients with wound cases at DMRH from February to April 2020(n = 242).

Variables	Characteristics	MRSA isolated N(%)	VRSA isolated N (%)
Sex	Male	16 (6.61)	5(2.07)
	Female	16 (6.61)	5(2.07)
Age (year)	< 15	3(1.24)	0(0)
	15–30	14(5.78)	3(1.24)
	31–45	8(3.31)	6(2.48)
	46–60	4(1.65)	1(0.41)
	> 60	3(1.24)	0(0)
Wound depth	Deep	19(7.85)	9(3.72)
	Superficial	13(5.37)	1(0.41)
Type of wound	Surgical wound	4 (1.65)	1(0.41)
	Non healing ulcer	4 (1.65)	1(0.41)
	Burn wound	3 (1.24)	0(0)
	Trauma	18 (7.44)	7(2.90)
	Abscess	2 (0.83)	1(0.41)
	Others	1(0.41)	0(0)
Total		32(13.22)	10(4.13)

Factors Associated with MRSA Prevalence

Socio-demographic characteristics of study participants were assessed for possible association with MRSA colonization (Tables 4). However, the association was not significant after adjusting for confounders using multivariate logistic regression ($p = 0.064$), sex has association with MRSA in bivariate analysis ($p = 0.005$).Participants residing in rural area were found to have higher number, 28.2% of MRSA isolates compared to urban dwellers, 16.9%. The association between residence and MRSA isolation was not statistically significant. Taken together, there was no significantly associated socio-demographic factors with MRSA.

Different clinical conditions assessed for possible association with MRSA infection. Depth of wound (p = 0.017), HIV (p = 0.027) and diabetes (p = 0.031), previous history of antibiotic use (p = 0.001), currently being on antibiotics (p = 0.004) and traditional medicine use (p = 0.034) showed statistically significant in univariate logistic regression analysis with MRSA colonization (Table 4).

To avoid the confounding effect, all variables with p-value of less than 0.2 were entered in to multivariate logistic regression analysis. As such, hospital stay > 72 h (p = 0.014, AOR: 7.402, 95% CI (1.502–39.49), previous history of wound infection (p = 0.001, AOR:24.33, 95% CI (3.77-156.89), currently being on antibiotics (p = 0.017; AOR = 7.9, 95% CI (1.44–43.39)), and depth of wound (p = 0.034, AOR = 4.38, 95% CI (1.049–18.29) still exhibited significant association with MRSA(Table 4).

Table 4
Binary logistic analysis of MRSA Prevalence and associated factors from wound cases at DMRH, February to April 2020, (n = 71).

Variable		MRSA N(%)	MSSA N (%)	COR(95% CI)	*p* value	AOR(95% CI)	*P*-value
Wound	Deep	19(26.8)	12(16.9)	3.29(1.23–8.76)	0.017	4.38(1.05–18.29)	0.043
	Superficial	13(18.3)	27(38.0)	1		1	
Hospital stay > 72 h	Yes	27(38.1)	17(24.0)	6.99(2.22–21.96)	0.001	7.40(1.5-39.49)	0.014
	No	5(7.0)	22(31.0)	1		1	
Previous wound infection	Yes	17(24.0)	5(7.0)	7.71(2.40-24.77)	0.001	24.3(3. 8-156.9)	0.001
	No	15(21.1)	34(47.9)	1		1	
On antibiotics	Yes	25(35.2)	7(9.8)	4.62(1.62–13.21)	0.004	7.9(1.44–43.39)	0.017
	No	17(24.0)	22(31.0)	1		1	

Note: COR = Crude Odds Ratio, AOR = Adjusted Odds Ratio, p = level of significance

Factors Associated with VRSA Prevalence

Similarly, we did analysis for digging out any variable that might be a predictor for VRSA. Despite, a wide 95%CI, deep site wound cases (p = 0.036; COR = 10.8, 95% CI (1.16-100.43)) and malignancy (p = 0.029; COR = 4, 95% CI (1.306-150.019)) revealed an alliance with VRSA, but not significant after adjusting for confounders using multivariate logistic regression (Table 5).

Table 5

Binary logistic analysis of VRSA Prevalence and associated factors from wound cases at DMRH, February to April 2020, (n=32)

Variable	VRSA	N (%)	(VISA+VSSA) N (%)	COR(95% CI)	*p*-value
Wound depth superficial	Deep	9(28.1)	10(31.25)	10.8(1.16-100.43)	0.036
		1(3.1)	12(37.5)	1	
Malignant	Yes	4(12.5)	1(3.1)	4(1.306-150.019)	0.029
Disease	No	6(18.8)	21(65.6)	1	
Note: COR= Crude Odds Ratio, *p*= level of significance					

Discussions

Staphylococcus aureus is cause to community and hospital acquired infections with high mortality rate in spite of the use of antibiotics. Although *S. aureus* is a part of normal human flora, it can cause a wide range of diseases, ranging from relatively mild skin infections to serious diseases. Many of these infections can rapidly become life-threatening if not treated and managed appropriately [2].

In this study 242 wound swab sample were processed and the proportion of *S. aureus* was 71/242 (29.3%). The prevalence of MRSA among the isolates were 45.1% which is above the national pooled prevalence data, 32.5% [12]. comparable to a systematic review and meta-analysis in Ethiopia at which the pooled prevalence of MRSA was 47% [10].Similar studies [11–16], also reported high proportion of MRSA at different corner of the country indicating that MRSA is becoming a challenging pathogen with limited choice of treatment in Ethiopia. Moreover, MRSA is an international health challenge as revealed by high rate of drug resistance report in different continents, 72% in Eritrea [17], 21% in Turky [18], 82.3% in Gaza Strip [19], 77.9% in Iran [20] and 76% tertiary care hospital in Lahore [21].

From the total isolate, 10(14. 1%) was VRSA which is inline with a systematic review and meta- analysis in Ethiopia the pooled prevalence of VRSA was 11%, 95% CI: (4–20) [10]. The prevalence of VRSA among MRSA was 10(31.3%) in the present study which was in agreement with studies elsewhere, (29.4%) [22] and (21.1%) [23]. Similar finding also reported in several countries around the world [8, 10, 24].Based on a large meta-analysis, the pooled prevalence of VRSA in Asia, Europe, America and Africa was 1.2%, 1.1%, 3.6% and 2.5%, respectively [24].

Treatment failure has been incriminated as a cause of decreased susceptibility of *S. aureus* to vancomycin[25]. In the present study with cut of value of MIC of MRSA ≥ 4 µg/ml E-test attained a resistance proportion of 65.6% 95% CI (46.9–81. 3) (VISA &VRSA). With this cut of value, our finding becomes comparable with several countries [8, 24, 25].

Previous publications have suggested that patients with intravenous drug use or cirrhosis were at higher risk for MRSA and VRSA carriage [26]. In the same manner, this study observed an association between

on antibiotics ($p = 0.017$). Deep wound infection ($p = 0.043$), previous wound infection ($p = 0.006$) and hospital stay > 72 h ($p = 0.001$) also demonstrated an association with MRSA infection. Unlike our study a studies by Shariati, (2020) found an association between several variables (hemodialysis dependence, long-term use of vancomycin, hospitalization in ICU and use of indwelling devices) [27]. Apparent absence of association between variables and VRSA in the present study might be due to small sample size.

Conclusions

In this study there was a higher prevalence of MRSA and VRSA among the study participants. Majority of MRSA strain was either VISA or VRSA strain; which has vancomycin MIC ≥ 4 µg /ml. Additionally, multiple variables has association with MRSA; such as previous wound infection, long time hospital stay, previous antibiotic usage and long-standing deep chronic wounds. Periodic surveillance and antibiotics stewardship program should be in place which can help generate data for empirical treatment. Further, genotypic studies are also needed to establish and characterize resistant strains of *S. aureus.*

Abbreviations

APHI, Amhara Public Health Institute; BDU, Bahir Dar University; CLSI, Clinical and Laboratory Standards Institute; DMRH, Debre Markos Referral Hospital; MHA, Muller Hinton Agar; MIC, minimum inhibitory concentration; MRSA, Methicillin Resistant *Staphylococcus aureus*; MSSA, Methicillin Sensitive *Staphylococcus aureus*; SCCmec, staphylococcal cassette chromosome mec; VISA, Vancomycin intermediate *Staphylococcus aureus*; VRSA, Vancomycin Resistant *Staphylococcus aureus*; VSSA, Vancomycin Sensitive *Staphylococcus aureus*;

Declarations

Acknowledgments

I would like to express my deepest gratitude to BDU, College of Medicine and Health Sciences, Department of Medical Laboratory Sciences for providing the opportunity to conduct M.Sc research project. The same special thanks go to Amhara National Regional State Health Bureau and DMRH for financial support. My special appreciation goes to APHI for providing me quality control strains. Lastly, my countless acknowledgements go to the study participants without them this study couldn't be realized.

Availability of data and materials

Almost all data generated and analyzed during this study were included in the manuscript. But if the spreadsheet dataset and full paper is needed, it will be shared upon request by the editor from the corresponding author.

Ethics approval and consent to participate

The study was approved by research and ethical review committee of Department of Medical Laboratory Sciences of BDU. Informed written consent was also obtained from each participant before data collection. All the information obtained from the study subjects were coded to maintain confidentially.

References

1. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of medical microbiology. 2015;64(4):323-34.
2. Bishara J, Goldberg E, Leibovici L, Samra Z, Shaked H, Mansur N, et al. Healthcare-associated vs. hospital-acquired *Staphylococcus aureus* bacteremia. International Journal of Infectious Diseases. 2012;16(6):e457-e63.
3. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. The Lancet. 2010;375(9725):1557-68.
4. Tomasz A. The staphylococcal cell wall. Gram-Positive Pathogens, Second Edition: American Society of Microbiology; 2006. p. 443-55.
5. D'Agata EM, Webb GF, Horn MA, Moellering RC, Ruan S. Modeling the invasion of community-acquired methicillin-resistant *Staphylococcus aureus* into hospitals. Clinical Infectious Diseases. 2009;48(3):274-84.
6. Zhu W, Murray PR, Huskins WC, Jernigan JA, McDonald LC, Clark NC, et al. Dissemination of an Enterococcus Inc18-Like vanA plasmid associated with vancomycin-resistant *Staphylococcus aureus*. Antimicrobial agents and chemotherapy. 2010;54(10):4314-20.
7. Anagaw B, Shiferaw Y, Anagaw B, Biadglegne F, Moges F, Kassu A, et al. Frequency of Methicillin-resistant *Staphylococcus aureus* isolates from Clinical Specimens in Gondar University Hospital, Northwest Ethiopia. Asian Journal of Medical Sciences. 2013;5(3):59-64.
8. Saeed, A., Ahsan, F., Nawaz, M., Iqbal, K., Rehman, K. U., & Ijaz, T. (2020). Incidence of Vancomycin Resistant Phenotype of the Methicillin Resistant *Staphylococcus aureus* Isolated from a Tertiary Care Hospital in Lahore. *Antibiotics,* **9**(1), 3.
9. Alani HA, Hassawi DS, Flayih MT. Patterns of Antibiotic Resistance in *Staphylococcus aureus* Isolates and Detection the Heteroresistance to Vancomycin by Population analysis Method. Journal of university of Anbar for Pure science. 2017;11(3):26-33.
10. Deyno S, Fekadu S, Astatkie A. Resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia: a meta-analysis. Antimicrobial Resistance & Infection Control. 2017;**6**(1):85.
11. Dilnessa T, Bitew A. 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. American Journal of Clinical and Experimental Medicine. 2016;4(1):7-12.
12. Eshetie S, Tarekegn F, Moges F, Amsalu A, Birhan W, Huruy K. Methicillin resistant *Staphylococcus aureus* in Ethiopia: a meta-analysis. BMC infectious diseases. 2016;16(1):689.

13. Kahsay A, Mihret A, Abebe T, Andualem T. Isolation and antimicrobial susceptibility pattern of *Staphylococcus aureus* in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. Archives of public Health. 2014;72(1):16.
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(1):11.
15. Mama M, Aklilu A, Misgna K, Tadesse M, Alemayehu E. Methicillin-and Inducible Clindamycin-Resistant *Staphylococcus aureus* among Patients with Wound Infection Attending Arba Minch Hospital, South Ethiopia. International journal of microbiology. 2019;2019.
16. Tadesse S, Alemayehu H, Tenna A, Tadesse G, Tessema TS, Shibeshi W, et al. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from patients with infection at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. BMC Pharmacology and Toxicology. 2018;19(1):24.
17. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients—A Multicenter Study in Asmara, Eritrea. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019;2019.
18. Rağbetli C, Parlak M, Bayram Y, Guducuoglu H, Ceylan N. Evaluation of antimicrobial resistance in *Staphylococcus aureus* isolates by years. Interdisciplinary perspectives on infectious diseases. 2016;2016.
19. El Aila NA, Al Laham NA, Ayesh BM. Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at Al Shifa hospital in Gaza Strip. BMC infectious diseases. 2017;**17**(1):28.
20. Emaneini M, Beigverdi R, van Leeuwen WB, Rahdar H, Karami-Zarandi M, Hosseinkhani F, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* isolated from burn patients in Iran: A systematic review and meta-analysis. Journal of global antimicrobial resistance. 2018;12:202-6.
21. Saeed A, Ahsan F, Nawaz M, Iqbal K, Rehman KU, Ijaz T. Incidence of vancomycin resistant phenotype of the methicillin resistant *Staphylococcus aureus* isolated from a tertiary care hospital in Lahore. Antibiotics. 2020;**9**(1):3.
22. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC infectious diseases. 2016;16(1):398.
23. Godebo G, Kibru G, Tassew H. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Annals of clinical microbiology and antimicrobials. 2013;12(1):17.
24. Shariati A, Dadashi M, Moghadam MT, van Belkum A, Yaslianifard S, Darban-Sarokhalil D. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate Staphylococcus aureus clinical isolates: a systematic review and meta-analysis. Scientific reports. 2020;10(1):1-16.

25. Kelley PG, Gao W, Ward PB, Howden BP. Daptomycin non-susceptibility in vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous-VISA (hVISA): implications for therapy after vancomycin treatment failure. Journal of antimicrobial chemotherapy. 2011;66(5):1057-60.
26. Harinstein L, Schafer J, D'Amico F. Risk factors associated with the conversion of meticillin-resistant *Staphylococcus aureus* colonisation to healthcare-associated infection. Journal of Hospital Infection. 2011;79(3):194-7.
27. Albrecht, V. S., Zervos, M. J., Kaye, K. S., Tosh, P. K., Arshad, S., Hayakawa,*et al* (2014). Prevalence of and risk factors for vancomycin-resistant *Staphylococcus aureus* precursor organisms in Southeastern Michigan. *Infection Control & Hospital Epidemiology,* **35**(12), 1531