INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

PRESENCE OF VANCOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAIN AND ANTIBIOTIC RESISTANCE PROFILE OF ISOLATES FROM A FEDERAL TEACHING HOSPITAL IN SOUTHEASTERN NIGERIA

Victor U. Usanga[1], Boniface N. Ukwah[1], Simon O. Azi[1], Michael E. Kalu[1*], Ugomma Ude[1]
[1] Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria.
* Corresponding author: Michael Kalu (kalu.erem@ebsu.edu.ng; +2348064028592)

ABS**TRACT**
The discovery of *Staphylococcus aureus (S. aureus)* which are resistant to vancomycin, threatens the relief had on vancomycin as the antibiotics of choice for the treatment of infections caused by methicillin resistant *Staphylococcus aureus*. The study was aimed at determining the prevalence of vancomycin-resistance *Staphylococcus aureus* (VRSA) and antibiotic resistance profile of isolates from various anatomical sites of patients attending Alex Ekwueme Federal Teaching Hospital, Ebonyi. A cross-sectional prevalence study was carried out on 121 samples including sputum, ear swab, urine, oropharyngeal abscess, wound swab, throat swab, endocervical swab, nasal swab, blood, and semen randomly collected from admitted patients. *Staphylococcus aureus* was isolated and identified using standard bacteriological techniques. The susceptibility testing was done using Kirby-Bauer disc diffusion method. 51 *Staphylococcus aureus* isolates were recovered from the 121 samples. None of the isolates identified was resistant to vancomycin. Resistance to other antibiotics were 19 (19.6%) to Ampicillin, 9 (17.6%) to methicillin, 5 (9.8%) to norfloxacin, 4 (7.8%) to each of erythromycin, streptomycin and gentamicin, 2 (3.9%) to chloramphenicol as well as ciprofloxacin and 1 (1.9%) to rifampicin. Most of the isolates were resistant to more than one antibiotic. More *Staphylococcus aureus* were isolated from the male than the female patients. However, there was insignificant observable difference in their resistance to the antibiotics. The 0% prevalence of VRSA observed in the present study reveals an in-depth knowledge of drug interaction among the health caregivers as well as the awareness of the danger associated with self-medication by the patients.

KEYWORDS: Vancomycin-resistant *Staphylococcus aureus* (VRSA), Vancomycin, Clinical isolates, Antibiotics, Southeast, Nigeria.

INRODUCTION

Staphylococcus aureus (*S. aureus*), a Gram positive cocci, catalase positive and coagulase positive bacteria, has been recognized as an important cause of human diseases ranging from skin to systemic infections for over a century[1]. A global challenge faced in the therapy for *S. aureus* is the emergence and consistent increase of strain of *S. aureus* which is resistant to penicillin, macrolides, tetracycline and aminoglycosides[2]. *Staphylococcus aureus* is one of the most common causes of nosocomial infections, especially pneumonia, surgical site infection as well as systemic infections, it continues to be a major cause of community-acquired infections. The battle against *S. aureus* remains a worry worldwide due to its ability to adapt to different environments, even to cope with antibiotic pressure[3]. Methicillin-resistance *S. aureus* (MRSA) was first detected approximately 40 years ago and remain in the list of priority 2 (high) bacteria to which antibiotics are urgently needed[4,5]. With the misuse of antibiotics, methicillin-resistant *S. aureus* strain became more prevalent, exceeding 70% of all *S. aureus* in Asia[6-8], and account for the majority of *S. aureus* infection with its consequent increased morbidity and mortality[9,10]. The therapy for MRSA infections began to shift to the use of glycopeptide antibiotics in the 1980s, particularly vancomycin which was considered to be an alternative drug for the treatment of MRSA[11]. Vancomycin is effective mostly against Gram-positive bacteria[11], where it acts by mitigating proper cell wall synthesis by forming a bond with the terminal D-alanyl-D-alanine moiety of the nascent cell wall lipid, thereby triggering the decomposition of the cell wall and consequently causing lysis of the bacteria[12,13]. Most Gram-negative bacteria are intrinsically resistant to vancomycin because their outer membrane is impermeable to glycopeptides molecules[13]. *Staphylococcus aureus* resistance to vancomycin had long been suspected in the clinics[12], but its reliance as an alternative to methicillin beclouded the fear of future problem. However, in 1997 it generated a significant concern in the medical community after its isolation in Japan and the United States[14,15].

The evolution of microbial resistance to vancomycin is a rising problem, especially within health care facilities such as hospitals. While newer alternatives to vancomycin exist, such as linezolid and daptomycin, the widespread use of vancomycin makes resistance to the drug a significant worry, especially in patients where resistant infections are not quickly identified and the patients continue the ineffective treatments[16]. An increase in vancomycin-intermediate and resistance *S. aureus* have been reported in many countries[6,17]. Initially, vancomycin-intermediate *S. aureus* (VISA) noted in Japan in 1996 and subsequently in the United States in 1997 was believed to be due to the thickened cell wall[2] where many vancomycin

molecules were trapped. The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules[18]. The second noted in the United States in 2002[19] among *S. aureus*, was identical to the mechanism seen in vancomycin-resistant enterococci[20].

Vancomycin–resistant *Enterococcus faecium* harbors the van A operon, which contains five genes, van S, -R, -H, -A and –X [23]. Further isolation of VISA and VRSA isolates from different countries[6,17,21,22] has confirmed that the emergence of this strain is of global concern. The increasing reports of *S. aureus* which are resistant to vancomycin as well as several currently accessible antibiotics has compromised treatment options with its consequent increased morbidity and mortality[23]. Therefore, the current study was carried out to determine the prevalence of vancomycin-resistant S. *aureus* and antibiotic resistance profile of isolates from a federal teaching hospital in southeastern Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 51 pure isolates of *S. aureus* were recovered from 121 samples including sputum, ear swab, urine, oropharyngeal abscess, wound swab, throat swab, endocervical swab, nasal swab, blood, and semen randomly collected from admitted patients in Alex Ekwueme Federal Teaching Hospital, Abakaliki between November 2018 and February 2019. The samples were immediately taken to Ultramodern Diagnostic Laboratory and Research Center, Ebonyi State University, where they were inoculated on media with minimum delay.

Processing of Samples

Sputum: The purulent part of the sputum was washed in sterile physiological saline and then the washed sputum was inoculated onto a blood agar plate and incubated at 37 ^{o}C for 24h.

Swabs: A sterile swab sticks were used to collect samples from wounds, throat, ear, nostril and cervix and inoculated onto plates of blood agar and incubated aerobically at 37 ^{o}C for 24h.

Urine: The urine samples (freshly collected clean-catch urine) were mixed by rotating the container. Then using a sterile wire loop, a loopful was plated on blood agar and inoculated aerobically at 37 ^{o}C for 24h.

Blood: 1ml of blood samples were collected and inoculated into 9ml of cooked meat broth and incubated at 37 ^{o}C for 48h, after which they were sub-cultured on blood agar plates and incubated aerobically at 37 ^{o}C for 24h.

Suspected isolates were purified by sub-culturing on mannitol salt agar and incubated at 37 ^{o}C for 24h, after which they were characterized by biochemical tests.

Isolation and Identification of *S. aureus*

The characterization of *S. aureus* isolates was carried out by Gram staining technique, catalase and coagulase tests, beta hemolysis of blood and sugar fermentation. The identification of *S. aureus* isolates was based on observed characteristics compared to the characteristics of reference *S. aureus* as published in reputable references particularly, Manual of Clinical Microbiology[23].

Antimicrobial susceptibility test

The modified disc diffusion method of Kirby and Bauer was used to determine the susceptibility of *S. aureus* isolates to the following antibiotics; Ciprofloxacin (10 µg), Gentamicin (10 µg), Streptomycin (30 µg), Rifampicin (20 µg), Erythromycin (30 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Vancomycin (30 µg), Norfloxacin **(**30 µg**)** and Methicilin (cefoxitin) (30 µg) from Oxoid Ltd, Hampshire, UK. Mueller-Hinton agar (Oxoid, Ltd., Hampshire, UK) medium was used for the test as recommended by the Clinical and Laboratory Standard Institute (CLSI)[24].

Preparation of inoculum and detection of vancomycin-resistant *S. aureus* (VRSA)

This was done using the colony suspension method. A colony of the overnight cultured isolate was picked with a sterile wire loop and inoculated into 0.5mL of sterile saline. This was incubated at 37^{o}C for 6 hours to achieve the same turbidity as 0.5 McFarland standard which is equivalent to 1.5 x 10^{6} CFU/mL[25,26]. Thereafter, a loopful of the bacterial suspension was inoculated onto the Mueller-Hinton agar plates, spread evenly to cover the surface. The antibiotic discs were placed aseptically on the inoculated agar plates and incubated at 37^{o}C for 24hr, after which the zones of inhibition were measured and the interpretation was done based on CLSI guidelines. A ≥15mm in diameter zone of inhibition of vancomycin was regarded as sensitive[27,28].

Statistical Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 20.0 (IBM SPSS Statistics 22, Chicago, IL, USA). Results were presented as frequency and percentages.

Ethical approval

Ethical approval was gotten from Ebonyi State University, Abakaliki management. Informed consents were signed by the participants after explaining the purpose and benefit of the study.

RESULTS

Out of the 51 pure isolates of *S. aureus* examined to determine the prevalence of vancomycin resistance and susceptibility to other antibiotics ranging from methicillin, ciprofloxacin, erythromycin, chloramphenicol, norfloxacin, streptomycin, rifampicin, Ampicillin and gentamicin, 12 were isolated from urine, 9 from wound swab, 8 from sputum, 5 from ear swab, 4 from throat swab as well as endocervical swab and blood, 2 each from semen, oropharyngeal abscess and 1 from nasal swab. Although none of the isolates was observed to be vancomycin-resistant, the antibiotic resistance profile showed that isolates from wounds had the highest prevalence of resistance to the antibiotics with the highest in ampicillin (44.4%). While isolates from sputum, blood, throat swab and ear swab showed minimal resistance to some of the antibiotics, isolates from urine, endocervical swab, nasal swab and semen were all susceptible to the antibiotics (Table 1 and figure 1).

TABLE 1: Prevalence of *S. aureus* and antibiotic resistance profile in relation to different types of sample

Types of specimen	Number of isolates	Prevalence (%) of Resistance									
		Cipr	Ery	Chlor	Norf	Strep	Met	Rif	Amp	Van	Gen
Sputum	8	0(0)	1(12.5)	0(0)	0(0)	1(12.5)	1(12.5)	0(0)	1(12.5)	0(0)	0(0)
Ear swab	5	0(0)	0(0)	1(20.0)	0(0)	1(20.0)	1(20.0)	0(0)	1(20.0)	0(0)	0(0)
Urine	12	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Oropharyngeal abscess	2	0(0)	0(0)	1(50.0)	1(50.0)	0(0)	1(50.0)	0(0)	1(50.0)	0(0)	1(50.0)
Wound swab	9	2(22.2)	2(22.2)	0(0)	2(22.2)	2(22.2)	3(33.3)	1(11.1)	4(44.4)	0(0)	1(11.1)
Throat swab	4	0(0)	0(0)	0(0)	0(0)	0(0)	1(25.0)	0(0)	1(25.0)	0(0)	1(25.0)
Endocervical swab	4	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Nasal swab	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Blood	4	0(0)	1(25.0)	0(0)	2(50.0)	0(0)	2(50.0)	0(0)	2(50.0)	0(0)	0(0)
Semen	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
TOTAL	51	2(3.9)	4(7.8)	2(3.9)	5(9.8)	4(7.8)	9(17.6)	1(1.9)	10(19.6)	0(0)	4(7.8)

Cipr = ciprofloxacin, Ery = erythromycin, Chlor = chloramphenicol, Norf = norfloxacin, Strep – streptomycin, Met = methicillin, Rif = rifampicin, Amp = Ampicillin, Van = vancomycin, Gen = Gentamicin

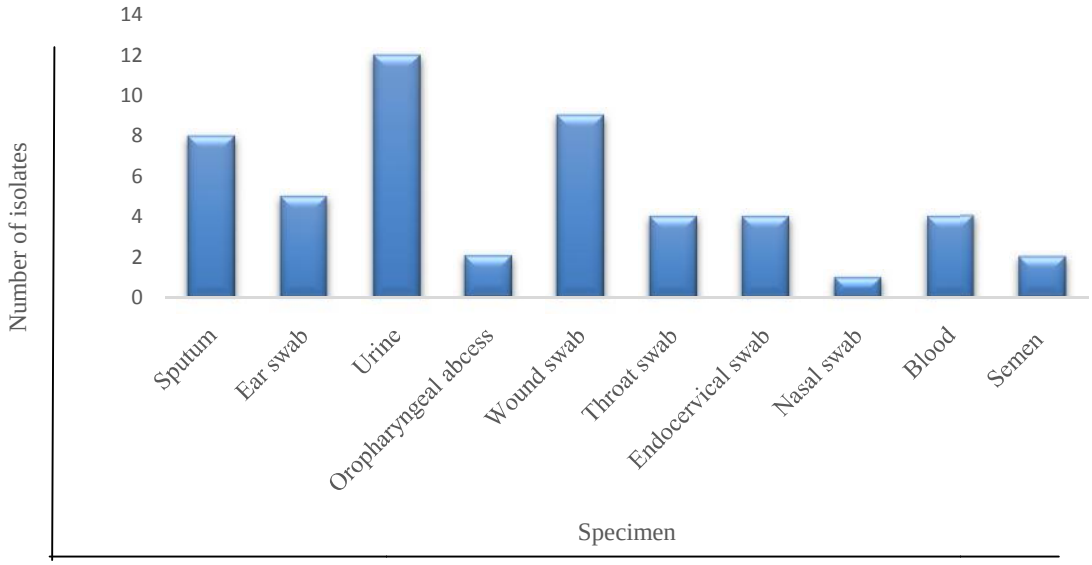

FIGURE 1: Prevalence of *S. aureus* isolates among the various specimen

TABLE 2: Prevalence of vancomycin and other antibiotics resistant *S. aureus* (n = 51)

Antibiotics	Susceptible		Resistant	
	Number	% Prevalence	Number	% Prevalence
Vancomycin	51	100	0	0
Methicilin	42	82.4	9	17.6
Chloramphenicol	49	96.1	2	3.9
Ciprofloxacin	49	96.1	2	3.9
Erythromycin	47	92.2	4	7.8
Norfloxacin	46	90.2	5	9.8
Streptomycin	47	92.2	4	7.8
Rifampicin	50	98.0	1	1.9
Ampicillin	41	80.4	10	19.6
Gentamicin	47	92.2	4	7.8

As shown in table 2, Ampicillin-resistant *S. aureus* (19.6%) had higher resistance prevalence compared to other antibiotics and was closely followed by methicillin-resistance *S. aureus* (17.6%). others were 9.8%, 7.8%, 7.8%, 7.8%, 3.9%, 3.9% and 1.9% for norfloxacin-resistant *S. aureus*, erythromycin-resistant *S. aureus*, streptomycin-resistant *S. aureus*, gentamicin-resistant *S. aureus*, chloramphenicol- resistant *S. aureus*, ciprofloxacin- resistant *S. aureus* and rifampicin-resistant *S. aureus* respectively. All the isolates were susceptible to vancomycin.

Higher number of isolates were recovered from the males than the females. While 29 (56.9%) of the total isolates were gotten from the male subjects, 22 (43.1%) were from the female patients. the antibiotics sensitivity profile of the isolates indicated that 2 (100%) and 0 (0%) isolates from the males and females respectively were resistant to ciprofloxacin, 3 (75.0%) and 1 (25.0%), was resistant to erythromycin, 0(0%) and 2 (100%) was resistant to chloramphenicol, 3 (60.0%) and 2 (40.0%) was resistant to norfloxacin, 2 (50.0%) and 2 (50.0%) was resistant to streptomycin, 5 (55.6%) and 4 (44.4%) was resistant to methicillin, 0 (0%) and 1 (100%) was resistant to rifampicin, 5 (50.0%) and 5 (50.0%) was resistant to Ampicillin, 0 (0%) and 0 (0%) was resistant to vancomycin, and 1 (25.0%) and 3 (75.0%) was resistant to gentamicin (Table 3).

TABLE 3: Prevalence of antibiotic resistance S. aureus in Relation to Gender

Gender	Number of isolates (%)	Prevalence (%) of resistance									
		Cipr	Ery	Chlor	Norf	Strep	Met	Rif	Amp	Van	Gen
Male	29 (56.9)	2(100.0)	3(75.0)	0(0)	3(60.0)	2(50.0)	5(55.6)	0(0)	5(50.0)	0(0)	1(25.0)
Female	22 (43.1)	0(0)	1(25.0)	2(100.0)	2(40.0)	2(50.0)	4(44.4)	1(100.0)	5(50.0)	0(0)	3(75.0)
Total	51 (100)	2(100)	4(100)	2(100)	5(100)	4(100)	9(100)	1(100)	10(100)	0(0)	4(100)

Cipr = ciprofloxacin, Ery = erythromycin, Chlor = chloramphenicol, Norf = norfloxacin, Strep = streptomycin, Met = methicillin, Rif = rifampicin, Amp = Ampicillin, Van = vancomycin, Gen = Gentamicin

DISCUSSION

Staphylococcus aureus has been recurrently implicated in infections ranging from mild superficial skin lesions to deep-seated infections such as osteomyelitis and endocarditis[1], having several potential virulence factors which include expression of surface protein for colonization of host tissues, host tissue-damaging toxins and phagocytosis inhibiting factors amongst others[29]. *Staphylococcus aureus* is one of the most dreadful pathogenic organisms, being able to resist the effect of many antibiotics including methicillin and vancomycin[30]. It increased resistance to multiple antibiotics posed a challenge in the clinics, redirecting attention to the use of vancomycin in the treatment of infections caused by MRSA. However, recent publications have identified and documented *S. aureus* which is not only methicillin-resistant, but also vancomycin-resistant.

In the present study, all the isolates showed complete (100%) susceptibility to vancomycin, depicting that vancomycin is still an effective antibiotic to be relied on for the treatment of infections caused by *S. aureus*. This agrees with the report of Otobo *et al*.[31], who reported a zero resistance of *S. aureus* isolated within a university premise in Nigeria. The 0% resistance to vancomycin observed in this study may be attributed to the in-depth knowledge of drug interaction and interference among the health caregivers in the hospital, being a teaching hospital. This could have reduced the chances of drug abuse as drugs administered to the patients are dispensed at the hospital pharmacy. However, with regards to the contradictory reports of Alo *et al*.[32], Olajuyigbe *et al*.[33] and Elsayed *et al*.[34]. The observed 0% resistance may be attributed to improved healthcare services and awareness after the publication and recommendations as suggested by Alo *et al*.[32]. Relatively high resistance to most of the antibiotics used in the study was observed among isolates from wounds while those isolated from semen, nasal cavity, cervix, as well as urine were susceptible to all the antibiotics in the study. Isolates from sputum, ear, oropharyngeal abscess, blood showed minimal resistance to a few of the antibiotics. Similar research carried out in a tertiary hospital in Kano, Nigeria, also revealed that more *S. aureus* (30.7%) were isolated from wounds than any other specimen[35]. Among the commonly isolated bacterial from severe wounds are *S. aureus* and *Pseudomonas aeruginosa*. Careful management of these organisms has been advocated due to their ability to develop resistance to multiple antibiotics[36]. Hospital environment poses a risk of acquiring infections and wounds are a risk factor for colonization by methicillin-resistant *S. aureus* as well other multidrug-resistant bacterial and this increases the severity of wounds and delays healing[36-38]. The prevalence of antibiotic resistance concerning the different samples showed that there was a higher prevalence in wound swab samples when compared to other samples[39].

The resistant patterns of the isolates to the various antibiotics in the present study were found to be highly variable. This is in agreement with global reports of an increasing antibiotics resistance to *S. aureus*[31]. This could be attributed to the self-medication of antibiotics mixture and indiscriminate use of antibiotics by patients. Other contributory factors may be the prolonged stay in the hospital as most people get infected in healthcare facility and such bacterial quickly develop resistance to several antibiotics, including beta-lactam[40]. Similar studies, showing the multi-drug resistance of nosocomial *S. aureus* carried out within Nigeria, showed similar results[31,32]. However, Alo *et al*.[32] reported the existence of VRSA among the sample, the studies included out-patients who visited the hospital. There was a slight difference in the prevalence of the *S. aureus* between the males and the females. The preponderance of the isolates was observed among the male patients when compared to the female. A similar not significant difference in the prevalence of *S. aureus* between males and females has been reported in previous studies[41-43].

CONCLUSION

The 0% prevalence of VRSA reported in the present study does not discredit reports indicating the presence of VRSA in the areas of study. However, it may reflect the healthcare givers' level of awareness of antibiotics resistance development due to improper administration of antibiotics, as well as encourage the use of vancomycin in sensitivity testing of *S. aureus* and it probable use for treatment of *S. aureus* infections. A limitation to the study was the inability of the authors to identify and detect resistance by molecular methods due to unavailability of

PCR/RT-PCR machine or the high cost of the test where available.

REFERENCES

[1]. Lowy FD. Staphylococcus infections. *New England Journal of Medicine* 2008; 339:520-532. DOI: 10. 1056 /NEJM199808203390806

[2]. Marananet MC, Moreire B, Boyle-Vavra S, Daum RS. Antimicrobial resistance in Staphylococci: Epidemiology, Molecular mechanisms and clinical relevance. *Infectious Disease Clinical of North America* 2007;**11**:813-849. doi.org/ 10.3109 /inf. 1983. 15.issue-4.05

[3]. Projan SJ. Antibiotics resistance in staphylococci: Gram positive pathogens. American Society for Microbiology, Washington, DC, 2002:463-470.

[4]. Deresinki S. Methicilin-resistant *Staphylococcus aureus* an Evolution, Epidemiologic and Therapeutic Odyssey. *Clinical Infectious Disease* 2005; **40**:562-573. DOI: 10.1086/427701

[5]. World Health Organisation. Who publishes list of bacteria for which new antibiotics are urgently needed. Geneva. 2017. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. Accessed 12/02/2019

[6]. Lee K, Chang CL, Lee NY, Kim HS, Hong KS et al. Korean nationwide surveillance of antimicrobial resistance of bacteria in 1998. Yonsei Medical Journal 2000; **41**:497-506. DOI 10.3349/ ymj.20 00.41.4.497

[7]. Voss A, Boebbeling BN. The worldwide prevalence of methicilin resistant *Staphylococcus aureus*. *International Journal of Antimicrobial Agents* 1995;**5**:101-106. DOI: 10.1016/0924-8579(94) 000 36-t

[8]. Whitener CJ, Park SY, Browne FA, Parent lJ, Fridkin SK. Vancomycin-resistant *Staphylococcus aureus* in the absence of vancomycin exposure. *Clinical Infectious Disease* 2004;**38**:1049-1055. DOI: 10.1086/382357

[9]. Jarvis WR, Schlosser J, Chinn RY, Tweeten S, Jackson M. National prevalence of methicillin-resistance Staphylococcus aureus in inpatients at US health care facilities, 2006. *Am J. infect Control*. 2007;**35**(10):631-737. DOI: 10.1016/j.ajic.2007.10.009

[10]. Klein E, Smith DL, Laxminarayan R. hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United State. 1999-2005. *Emerg Infect Dis* 2007;**13**(12):1840. doi: 10.3201/eid1312.070629

[11]. Moellering RC Jr,. Vancomycin: a 50-year reassessment. *Clin Infect Dis*. 2006; **42**(1):S3-S4. DOI:10.1086/491708

[12]. Lechevalier MD. Prauser H, Labeda DP, Ruan JS. Two new genera of nocardioform actinomycetes: Amycolata gen. nov. and Amycopatopsis gen. nov. *Int. J. Syst. Bacteriol* 1986; **36**:29-37. DOI.Org/10. 1099/00207713-36-1-29

[13]. Geraci JE, Wilson WR. Vancomycin therapy for ineffective endocarditis. *Review of Infectious Disease* 1981;5250-5258. DOI: 10.12659/ajcr.902751

[14]. Moellering RC. The specter of glycopeptide resistance: current trends and future considerations. *Am. J. Med* 1998;**104**(5A):3S–6S. DOI.Org/ 10.10 16/ S0002-9343(98)00148-X

[15]. Hiramatsu Y, Hosoda S. Dissemination in Japanese Hospitals of strains of S. aureus heterogeneously resistant to vancomycin. *Lancet* 1997;**350**:1670-1673. DOI:https://doi.org/10.1016/S0140-6736 (97) 07324-8

[16]. Benjamin PH, John KD, Paul DR, Timothy PS, Grayson ML. Reduced Vancomycin Susceptibility in *Staphylococcus aureus*, Including Vancomycin-Intermediate and Heterogenous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clnical Implications. *Clin Microbiol Rev* 2010;**23**(1):99-139. doi: 10.1128/ CMR.00042-09

[17]. Cui L, Iwamoto A, Lian JQ, Neohm HM, Maruyama T *et al*. Novel mechanism of antibiotic resistance originating in vancomycin- intermediate *Staphylo coccus aureus. Antimicrobial Agents and Chemotherapy* 2006; **50**:428-438. **DOI:** 10.11 28/ AAC.50.2.428-438.2006

[18]. Bozdogan BU, Esel D, Whitener C, Browne FA, Appelbaum PC. Antimicrobial susceptibility of a vancomycin-resistant *Staphylococcus aureus* strain isolated at the Hershey Medical Center. *Journal of Antimicrobial Chemotherapy* 2003;**52**:864-868. DOI: 10.1093/jac/dkg457

[19]. Authur M, Molinas C, Departdieu F, Courvalin P. Characterization of Tn1546, a Tn3-Related transposons conferring glycopeptides resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium. Journal of Bacteriology* 1993; **175**:117-127. doi: 10.1128/jb.175.1.117-127.1993

[20]. Tiwari HK, Sen MR. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. *Infectious Diseases* 2006;**6**:156-159. DOI: 10.1186/ 1471-2334-6-156

[21]. Pina P, Vandenesch F, Bedos JP, Allovel PY. An outbreak of *staphylococcus aureus* strains with reduced susceptibility to Glycopeptides in a French general Hospital. *Clinical Infectious Disease* 2000; **31**:1306-1308. DOI: 10.1086/317464

[22]. Arjun S, James DD, Trish MP. Vancomycin Resistance in Staphylococci. Clinical Microbiology Reviews 2002; **15**(3):430-438. DOI: 10.1128/ CMR.15.3.430–438.2002

[23]. Gary PW, Bruce AH. Manual of Clinical Microbiology (8th edition) Edited by Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Michael A. Pfaller, and Robert H. Yolken. Washington DC: ASM press 2004; 1199-1200. doi.org/10.1086/383067

[24]. Clinical and Laboratory Standards Institute (CLSI). Performance standards for anti-microbial disk susceptibility test. 13th ed. CLSI standard M02. Wayne, PA. Clinical and Laboratory Standards Institute, 2018.

[25]. Ferraro MJ, Craig WA, Dudley MN, Eliopoulos GM, Hecht DW *et al*. Performance standards for antimicrobial disk susceptibility tests. Approved Standard M2-A7. 7th ed. Wayne (PA): National Committee for Clinical Laboratory Standards; 2000.

[26]. Bauer AW, Kirby WMM, Sherris JC, Tierch M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol* 1968;**45**: 493e6. doi.org/10.1093/ajcp/45.4_ts.493

[27]. Kaiser ML, Thompson DJ, Malinoski D, Lane C, Cinat ME. Epidemiology and risk factors for hospital-acquired methicillin-resistant Staphylo coccus aureus among burn patients. *J Burn Care Res* 2011;**32**(3):429–434. doi.org/10.1097/ BCR.0b013 e318217f92d

[28]. Schweizer M, Ward M, Cobb S, McDanel J, Leder L *et al*. The epidemiology of methicillin-resistant Staphylococcus aureus on a burn trauma unit. *Infect Control Hosp Epidemiol* 2012;**33**(11):1118–1125. DOI: 10.1086/668032

[29]. Easmon CSF, Adlam C. Staphylococci and staphylococcal infections. Vol. 1 & 2. Academic Press, London 1983.

[30]. Friaes A, Resina C, Manuel V, Lito L, Ramirez M, *et al*. Epidemiological survey of the first case of vancomycin-resistant Staphylococcus aureus infection in Europe. *Epidemiol Infect* 2015; 1**43**: 745e8. DOI: 10.1017/S0950268814001423

[31]. Otobo NU, Wala PG, Agbagwa OE. Occurrence of Vancomycin-Resistant *Staphylococcus Aureus* (Vrsa) in Clinical and Community Isolates Within the University of Port Harcourt. *Advances in Biotechnology and Microbiology*. 2018;**11**(3): AIBM.ID.555816
DOI: 10.19080/AIBM.2018.11.555816

[32]. Alo M, Ugah U, Okoro N. Epidemiology of Vancomycin Resistant Staphylococcus Aureus among Clinical Isolates in a Tertiary Hospital in Abakaliki, Nigeria. American Journal of Epidemiology and Infectious Disease. 2013; **1**(1):24-26. DOI:10.12691/AJEID-1-3-2

[33]. Olajuyigbe O, Ikpehae T, Coopoosamy R. Multidrug and vancomycin resistance among clinical isolates of Staphylococcus aureus from different teaching hospitals in Nigeria. *African Health Science.* 2017; **17**(3):797-807. doi: 10.4314/ahs.v17i3.23

[34]. ElSayed N, Ashour M, Amine AEK. Vancomycin resistance among Staphylococcus aureus isolates in rural setting, Egypt. *GERMS*. 2018;**8**(3): 134-139. doi: 10.18683/germs.2018.1140

[35]. Nwankwo EO, Nasiru MS. Antibiotic sensitivity pattern of Staphylococcus aureus from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. *The Pan African Medical Journal*. 2011;8:4. doi:10.4314/pamj.v8i1.71050

[36]. Albaugh KW, Biely SA, Cavorsi JP. The effect of a cellulose dressing and topical vancomycin on methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-positive organisms in chronic wounds: a case series. *Ostomy Wound Manage*. 2013;**14**(5):34–43. PMID: 23669259

[37]. McKinnell JA, Huang SS, Eells SJ, Cui E, Miller LG. Quantifying the impact of extranasal testing of body sites for methicillin-resistant Staphylococcus aureus colonization at the time of hospital or intensive care unit admission. *Infect Control Hosp Epidemiol*. 2013;**14**(2):161–170. doi: 10.1086/ 669 095.

[38]. Ramarathnam V, De Marco B, Ortegon A, Kemp D, Luby J *et al*. Risk factors for development of methicillin-resistant Staphylococcus aureus infection among colonized patients. *Am J Infect Control*. 2013; **14**(7):625–628. doi: 10.1016/j.ajic. 2012. 08.005

[39]. Ikeh EI. Methicilin-resistant Staphylococcus aureus (MRSA) at Jos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology* 2003;**4**(1):52-55. DOI: 10.4314/ ajcem. v4i1.7324

[40]. Song JH, Hiramatsu K, Suh JY, Ko KS, Ito T *et al*. Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Antimicrobial agents and chemotherapy* 2004; **48**:4926-4928. doi: 10.1128/AAC.48.12.4926-4928. 2004

[41]. Sulaiman A, AL Y, Sabry Y, Mahmoud E, Taha M. Prevalence of methicillin-resistant S*taphylococcus aureus* in Saudi arabia: systemic review and meta-analysis. *Afr J Cln Exp Microbiol* 2013;**14**(3): 146-154. doi.org/10.4314/ajcem.v14i3.5

[42]. Okwu M, Sinat B, Wakeel A. Prevalence of nasal carriage of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) among healthy primary school children in okada, Nigeria. J Nat Sci Res 2012;**2**(4): 61-67. Corpus ID: 30275230

[43]. Ankur B, Devjyoti M, Barnali P. Nasal carriage of methicillin resistant staphylococci in healthy population of East sikkim. *Indian J Community Med* 2009; **34**(4):364-365. doi: 10.4103/0970-0218.58403