SJIF Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 8.
Issue: 12
Page N. 13-21
Year: 2024

Original Article www.wjahr.com

PREVALENCE AND ANTIBIOTIC RESISTANCE PROFILE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS OBTAINED FROM CLINICAL SPECIMENS IN FEDERAL MEDICAL CENTRE MAKURDI, BENUE STATE

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Article Received date: 25 September 2024 **Article Revised date:** 15 October 2024 **Article Accepted date:** 05 November 2024

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ABSTRACT

Background: The emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) has caused a new level of threat to microbial infection control and antimicrobial therapy. **Aim:** To determine the prevalence and antibiogram of Methicillin resistant Staphylococcus aureus (MRSA) isolated from clinical specimens in the Federal Medical Centre Makurdi, Nigeria. **Methods:** A total of 86 *S. aureus* isolates were recovered from 400 clinical specimens. *S. aureus* isolates were identified by growth and fermentation on mannitol salt agar, Gram stain, and positive results for catalase and tube coagulase tests. The 86 *S. aureus* isolates were tested for susceptibility to antibacterial agents by the disc diffusion method. *S. aureus* isolates that were resistant to cefoxitin were regarded as MRSA. PCR was used to confirm MRSA by amplification of the *mecA* gene. **Results:** Fourteen (16.3%) of the 86 *S. aureus* isolates were resistant to cefoxitin and positive for *mecA* gene by PCR. Most of the MRSA isolates were cultured from wound 7 (8.1%) and blood 3 (3.5%) specimens. Majority of MRSA isolates were recovered from males 11 (18.6%) patients and age group 1-17 years 2 (33.3%) and 18-33 years 8 (18.6%). All fourteen (100.0%) MRSA isolates were resistant to penicillin and trimethoprim. Also, high rate of resistance was observed in ciprofloxacin 13/14 (92.8%) and tetracycline 10/14 (71.4%). **Conclusion:** The study has revealed MRSA prevalence of 16.3% and provided a platform for future studies on MRSA in the study area.

KEYWORDS: Antibiogram, Polymerase chain reaction, MRSA.

INTRODUCTION

Methicillin Resistant Staphylococcus aureus evolved through the acquisition of the *mecA* gene by previously susceptible isolates. The *mecA* gene is responsible for the synthesis of a novel penicillin-binding protein known as penicillin-binding protein 2a, which has decreased binding affinity for penicillin and cephalosporins and therefore confers resistance to beta-lactam antibiotics except the 5th generation cephalosporin, Ceftaroline (ALFouzan *et al.*, 2020).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare and community-associated infections worldwide (Abdel-Maksoud *et al.*, 2016; Adam and Abomughaid, 2018). Since its emergence in UK in 1960s (Jevons, 1961), the frequency of isolation of MRSA has been on the increase worldwide, (Sit *et al.*, 2017; Lakhundi and Zhang, 2018), including Nigeria (Ayepola *et al.*, 2015; Nsofor *et al.*, 2016; Essien *et al.*, 2021).

It has been established that the epidemiology of MRSA strains is constantly changing in different geographical locations (Akanbi and Mbe, 2012; Yusuf and Airauhi, 2015; Essien *et al.*, 2021; Umoh *et al.*, 2024). It is essential to study MRSA isolates from local healthcare facilities to obtain local data that can be utilized for empirical treatment of infections and design appropriate infection control measures.

In Nigeria, the prevalence of MRSA isolates obtained from clinical specimens have been studied in the various geopolitical zones; Southwest (Ghebremedhin *et al.*, 2009; Raji *et al.*, 2013; Ogbolu *et al.*, 2015), Southsouth

(Azeez *et al.*, 2008; Yusuf and Airauhi, 2015; Umoh *et al.*, 2024), South East (Egbuobi *et al.*, 2014; Okoye *et al.*, 2022), Northeast (Okon *et al.*, 2013; Okon *et al.*, 2014) Northwest (Nwankwo *et al.*, 2010; Idri *et al.*, 2019) and Northcentral (Akanbi and Mbe, 2012; Essien *et al.*, 2021). However, there are limited data on prevalence of MRSA colonizing or infecting patients in Makurdi, North Central Nigeria.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethical Committee of the Federal Medical Centre Makurdi, Benue State (FMH/FMC/MED.108/VOL.1/X).

Bacterial isolates

S. aureus isolates were sourced from different clinical specimens obtained from patients attending Federal Medical Centre Makurdi, Benue State. The Federal Medical Centre is a 400-bed hospital situated in Makurdi, Benue State in the North-Central zone of Nigeria. A total of 86 *S. aureus* isolates were sourced from wounds, ear, catheters, urethra, and high vagina, urine, blood, aspirate, throat swab and cerebrospinal fluid. The *S. aureus* was identified by growth and fermentation on mannitol salt agar, Gram stain, and positive results for catalase and tube coagulase tests at the Department of Medical Laboratory Science, University of Jos, Nigeria.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed by the disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI, 2017). Pure colonies of an overnight culture of *S. aureus* on Brain Heart Infusion agar were suspended in normal saline and adjusted to a turbidity equivalent to 0.5 X MacFarland standard (1.5 x 10^8 CFU/ml). The suspension was poured on to Mueller-Hinton agar (MHA) (Oxoid, UK) plates and excess fluid was discarded and plates left to dry. Antibiotic discs were placed on the dried surface and the plates were incubated at 37°C for 24 hours. *S. aureus* strain, ATCC 25923, was used as control for sensitivity testing. The inhibition zone around each disc was measured and interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2017).

The following antibiotic impregnated discs were used for this study: penicillin (10 units), cefoxitin (30 µg), gentamicin (10 µg), erythromycin (15 µg), rifampicin (5 µg), Tetracycline (30 µg), trimethoprim (25 µg), Chloramphenicol (30 µg) and Ciprofloxacin (5 µg). Bacterial isolates resistant to cefoxitin (≤14mm) were regarded as MRSA (CLSI, 2017).

Molecular analysis of *S. aureus* isolates

Extraction of *S. aureus* DNA for PCR

DNA isolation was carried out according to the method described by (Udo *et al.*, 1999). Three to five identical colonies of an overnight culture were picked using a sterile loop and suspended in a microfuge tube containing 50 µl of lysostaphin (150 µg/ml) and 10 µl of RNase (10 µg/ml) solution. The tube was incubated at 37 °C in the heating block (Thermo Mixer, Eppendorf, Hamburg, Germany) for 20 min. To each sample, 50 µl of proteinase K (20 mg/ml) and 150 µl of Tris buffer (0.1 M) were added and mixed by pipetting. The tube was then incubated at 60 °C in the water bath (VWR Scientific Co., Shellware Lab, United States) for 10 min. The tube was transferred to a heating block at 95^{o}C for 10 min to inactivate proteinase K activity. Finally, the tube was centrifuged, and the extracted DNA was stored at 4 °C till used for PCR.

Preparation of agarose gel

Gel electrophoresis was used to separate DNA based on their sizes by applying an electric field to move the DNA through an agarose matrix. Two 2% (w/v) concentration of agarose gel used in the study was prepared by weighing and dissolving 4 grams of agarose powder (Promega, Madison, USA) in 200 ml Tris-Borate-EDTA (1XTBE buffer) (Gibco, UK) using microwaved oven. The mixture was heated in a microwave oven until it became clear and transparent. The molten agarose was allowed to cool to about 45oC and 300 µl (1mg/ml) of ethidium bromide was added to the gel. The molten gel was gently poured in a mould (casting) and was allowed to solidify after which the comb was removed to create wells for DNA sample application. The gel was transferred from the mould into an electrophoretic chamber (Bio-Rad, USA) filled with (1x TBE) buffer.

Detection of *mecA* gene by PCR

Methicillin resistance was determined by *mecA* PCR as described (Zhang *et al.*, 2005). Amplification of *mecA* gene was performed on all isolates resistant to cefoxitin. The total reaction volume 25 µL was used for PCR. This volume contained 2µl of genomic DNA, 12.5 µl of Hot Star Red Taq Master mix, 8.5µl PCR H_2O and 2µl each of *mecA* primers (Qiagen, Hilden, Germany). *mecA* gene was amplified with the following primers: *mecA*- F: (F-GTG AAG ATA TAC CAA GTG ATT); *mecA*-R (ATGCGC TAT AGA TTG AAA GGA T). DNA amplification was carried out for 40 cycles according to the following protocol: denaturation at 94° C for 30 s, annealing at 55° C for 30 s, and extension at 72° C for 1 min with a final extension at 72° C for 5 min. After amplification by PCR, 8 µl of each of PCR products were mixed with tracking dye and transferred accordingly into the wells of 2% (w/v) agarose gels. *mecA* positive DNA sample was included as control in every batch of the agarose run. The electrophoresis chamber was connected to the power source and the DNA was run at 120V for 30 minutes. The separated DNA fragments in the gel were transferred into a transilluminator and were visualized by illumination of UV light. The DNA bands were recorded by photography using computer system.

Statistical analysis

Data obtained from this study were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. The MRSA, gender, age-group, were compared using Pearson chi-square tests. Results were presented in tables, bar chart and percentages. P-values of <0.05 were considered statistically significant.

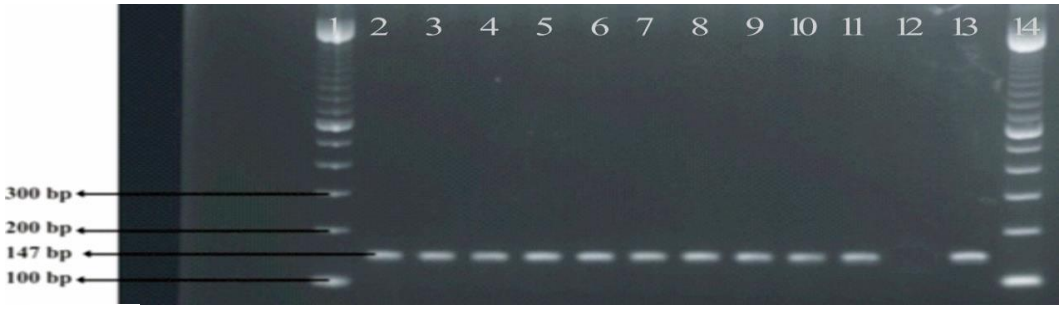

Figure 1: Agarose gel electrophoresis used for detection of amplified *mecA* gene by PCR.

Lanes 1 and 14 shows 100bp DNA molecular size ladder used for sizing DNA bands of test samples. The ladder consists of 100bp DNA bands ranging from 100 to 1500bp. Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 represent DNA bands from test samples that were positive for *mecA*. Lane 12 and 13 represent negative and positive mecA control samples respectively. The amplified *mecA* gene is 147 bp in size, therefore it was located between 100bp and 200bp.

RESULTS AND DISCUSSIONS

A total of 86 *S. aureus* were isolated from 10 different types of clinical specimens. Fourteen MRSA were detected among the *S. aureus* isolates, yielding an overall prevalence of 14/86 (16.3%). The distribution of MRSA isolates according to different specimens is summarized in Table1. Most of the MRSA isolates were recovered from wound swabs (7/86; 8.1%), followed by blood culture (3/84; 3.5%), urine, urethral, ear swab and CSF each produced one MRSA isolate (N=1; I.5%). Majority of MRSA isolates recovered from wound swab and blood culture. These results were similar to results of studies conducted in South-west, Nigeria (Ghebremedhin *et al.*, 2009; Alli *et al.*, 2015; Ayepola *et al.*, 2015), North-Central, Nigeria (Abdullahi and Iregbu, 2018), North-Eastern, Nigeria (Okon *et al.*, 2011, Akanbi and Mbe, 2012; Okon *et al.*, 2014) and South-South, Nigeria (Yusuf and Airauhi, 2015) that demonstrated the predominance of MRSA isolates in wound swabs and blood samples.

Table 1: Distribution of MRSA according to types of clinical specimens.

Specimens	No. of *S. aureus* isolates N=86	No. of MRSA N=14 (%)
Wound swab	46	7 (8.1)
Urine	14	1 (1.5)
Blood	10	3 (3.5)
Catheter	4	0 (0.0)
Urethral	4	1 (1.5)
HVS	3	0 (0.0)
Throat	2	0 (0.0)
Aspirate	1	0 (0.0)
Ear swab	1	1 (1.5)
CSF	1	1 (1.5)

Table 2. Indicates the distribution of MRSA with respect to gender and age group of patients. With regards to gender of patients, more MRSA 11/59 (18.6%) isolates were recovered in male patients compared to female patients 3/27 (11.1%). However, the differences in the number of MRSA isolates obtained from male and female patients was not statistically significant (P=0.380; P>0.05) which implies that gender was not a significant determining factor for MRSA infection in this study.

Most of the MRSA 3/6 (33.3%) and 8/43 (18.6%) isolates were obtained from patients within 1-17 years and 18-33 years respectively. This indicates that younger patients were at greater risk of infections caused by MRSA. This observation agreed with reports from similar previous studies in Nigeria (Abdullahi and Iregbu, 2018), Ethiopia (Dilnessa and Bitew, 2016) and Libya (Buzaid *et al.*, 2011). Statistically, there was no significant difference in the occurrence of MRSA isolates among the age group investigated (P>0.05; P=0.411).

Table 2: Distribution of MRSA in patients with different demographic backgrounds.

Gender	**No. of *S. aureus***		
Male	59	11(18.6)	0.380
Female	27	3 (11.1)	
Age group			
1-17	6	2 (33.3)	0.411
18-33	43	8 (18.6)	
34-50	31	4 (12.9)	
51+	6	0 (0.0)	

Figure 2. Reveals the resistant profile of the fourteen MRSA recovered in the study. Cefoxitin is a surrogate marker for methicillin resistance. Therefore, the 14 cefoxitin-resistant *S. aureus* isolates were considered methicillin resistant yielding a prevalence of 14/86 (16.3%). Methicillin resistance in these isolates were confirmed by the detection of *mecA* gene by PCR. All 14 cefoxitin-resistant strains were positive for *mecA.* Besides, all MRSA isolates were resistant to penicillin and trimethoprim (N=14;100.0%). Also, the rate of resistant was high for ciprofloxacin 13/14 (92.8%) and tetracycline 10/14 (71.4%). This was followed by gentamicin 7/14 (50.0%). Three isolates each were resistant to erythromycin and chloramphenicol 3/14 (21.4%). The least resistant 1/14 (7.1%) was observed in rifampicin.

The prevalence of MRSA (16.3%) observed in this study was similar to previous studies conducted on *S. aureus* by Ayepola *et al.,* (2015) who reported 15.5% prevalence in a multicenter study of *S. aureus* investigated in healthcare facilities in Southwest Nigeria. Also, studies that revealed higher prevalence have been reported in Nigeria; such as 22.6% from North Central (Mofolorunsho *et al.,* 2022), the 43.3% and 33.3% prevalence in Jos University Teaching Hospital, North Central Nigeria by Ikeh (2003) and Okwu *et al.,* (2014) respectively, 42.9% was reported by Bawonda *et al.,* (2024) at the University of Uyo teaching Hospital, and the 42.7% prevalence reported in University of Benin Teaching Hospital, South-South Nigeria by Yusuf and Airauhi, (2015). In addition, Nsofor *et al.,* (2016) reported that 38.5% of *S. aureus* obtained at Abia State University Teaching Hospital, Aba, Southeast Nigeria were MRSA.

Furthermore, studies outside Nigeria indicating higher MRSA prevalence were as follows; 47.0% by Deyno *et al.,* (2017) in Ethiopia, 42.5% by Pirko *et al.,* (2019) in Iraq and in Saudi Arabia (Adam and Abomughaid, 2018; 38%).

In contrast, the 16.3% prevalence reported in this study was higher than 13.1% reported among *S. aureus* obtained at the University of Abuja Teaching Hospital (Akanbi and Mbe, 2012) North Central Nigeria, Okon *et al.,* (2011) and Okon *et al.,* (2014) revealed MRSA prevalence of 12.5% and 8% respectively from Northeast Nigeria. These reports highlight differences in the prevalence of MRSA in different health facilities in Nigeria which may reflect differences in antibiotic use or infection control practices.

All the MRSA isolates in this study were resistant to trimethoprim. Trimethoprim resistance was also detected in 100% of MRSA isolates investigated in a hospital in Ethiopia (Dilnessa and Bitew, 2016). Elsewhere, 95% of MRSA isolates investigated in Pakistan (Akhter *et al.,* 2009) and 88.2% of MRSA reported from Uganda (Ojulong *et al.,* 2009) were trimethoprim resistant. The high rate of resistance to trimethoprim is cause for concern, since it can no longer be used for treating infections caused by MRSA.

Ciprofloxacin resistance was detected in 92.8% of the MRSA isolates in this study. Similarly, high prevalence of ciprofloxacin resistance was reported in MRSA studied in other parts of Nigeria; (Yusuf and Airauhi, 2015; 100%) in North Central Nigeria, (Ugbogu *et al.,* 2007; 58.8%) and (Abdullahi and Iregbu, 2018; 62.8%) in Southeastern and North Central Nigeria respectively. Similarly, studies conducted elsewhere have observed a higher and lower resistance to ciprofloxacin, Uganda (Ojulong *et al.,* 2009; 70.6%) and the 8.0% prevalence reported from Iraq (Mussa *et al.,* 2018). The high rate of resistance to ciprofloxacin observed in this and other studies implies that ciprofloxacin should not be used in the treatment of MRSA infections without the results of antibiotic susceptibility testing.

Tetracycline resistance was detected in 71.4 (10/14) of the MRSA isolates in this study. A study by Yusuf and Airauhi, (2015) reported tetracycline resistance in 100% of their isolates. Other studies by Abdullahi and Iregbu (2018), Olowe *et al.,* (2012), and Adetayo *et al.,* (2014) reported tetracycline resistance in 88.0%, 87.5 % and 57.1% respectively in different centres in Nigeria. Furthermore, studies on antibiotic resistance rates in South Africa (Amoako *et al.,* 2019) and Nepal (Shahi *et al.,* 2018) reported 62.67% and 33.3% respectively among MRSA that they investigated. The differences in the proportion of tetracycline-resistant MRSA strains in different studies may reflect differences in antibiotic use.

In this study, 7/14 (50.0%) of the MRSA isolates were resistant to gentamicin. Similar level of resistance to gentamicin (42.9%) was published by Adetayo *et al.,* (2014) from Southwest, Nigeria. Other studies detailing different prevalence of gentamicin resistance in MRSA in Nigeria include 100% by Yusuf and Airauhi, (2015),

62.5% by Olowe *et al.*, (2007), 53.6% by Abdullahi and Iregbu, (2018), 54.5% by Udobi *et al.*, (2013), 58.8% in Uganda (Ojulong *et al.*, 2009), 84.4% in Iran (Khosravi *et al.*, 2017), 55% in Pakistan (Akhter *et al.*, 2009) and 55.1% in India (Abdul *et al.*, 2017). In contrast, lower level (25.2%) of gentamicin resistant-MRSA was reported by Akpaka *et al.*, (2017) from Trinidad and Tobago and Cameroon (32.0%) (Njoungang *et al.*, 2015). Interestingly, all MRSA isolates investigated by Ghebremedhin *et al.*, (2009) were susceptible to gentamicin.

The 21.4 (3/14) resistance rate to erythromycin in this study was much lower than the 60.0% prevalence reported previously among MRSA isolated in Abuja-Nigeria (Abdullahi and Iregbu, 2018). Higher resistance rates of erythromycin resistance in MRSA were published in Ethiopia (100%; Dilnessa and Bitew, 2016), Pakistan (70%; Akhter *et al.*, 2009), India (63.2%; Rajaduraipandi *et al.*, 2006), Cameroon (50%; Njoungang *et al.*, 2015) and Turkey (58.1%; Hanci *et al.*, 2017).

Chloramphenicol resistance was detected in 21.4% (3/14) of the MRSA isolates in this study. Similarly, proportions of MRSA resistant to chloramphenicol; 28.4%, 24.1% and 33.1% were reported previously in Nigeria (Onelum *et al*, 2015), Pakistan (Fayyaz *et al.*, 2013) and Kaleem *et al.*, (2010) respectively. Resistance rates lower than what was obtained in this study were reported from Kuwait (8.2%) by Boswihi *et al.*, (2016), 4.8% in the Gambia by Darboe *et al.*, (2019), 16.75% from Iran by Sales *et al.*, (2018) and 19% in South Africa by Marais *et al.*, (2009). Higher resistance rates have been observed in other previous studies namely, 78.8% by Azeez-Akande *et al.*, (2008) and 70.1% Nsofor *et al.*, (2016) in Nigeria, 88.2% by Ojulong *et al.*, (2009) from Uganda and 41.6% by Shahi *et al.*, (2018) from India. These results highlight differences in the prevalence of chloramphenicol resistance in MRSA obtained in different geographic regions.

Resistance to rifampicin 1/14 (7.1%) was detected in small number of MRSA isolates indicating that these antibiotics is still active against most MRSA isolates. Similarly, low prevalence of resistance to rifampicin (6.4%) among MRSA isolates was observed in a study reported previously in Kano, North Western, Nigeria (Kumurya, 2015). Elsewhere, similar low resistant (6.2%) was reported among MRSA isolates by Yilmaz and Aslantas (2017) in Turkey.

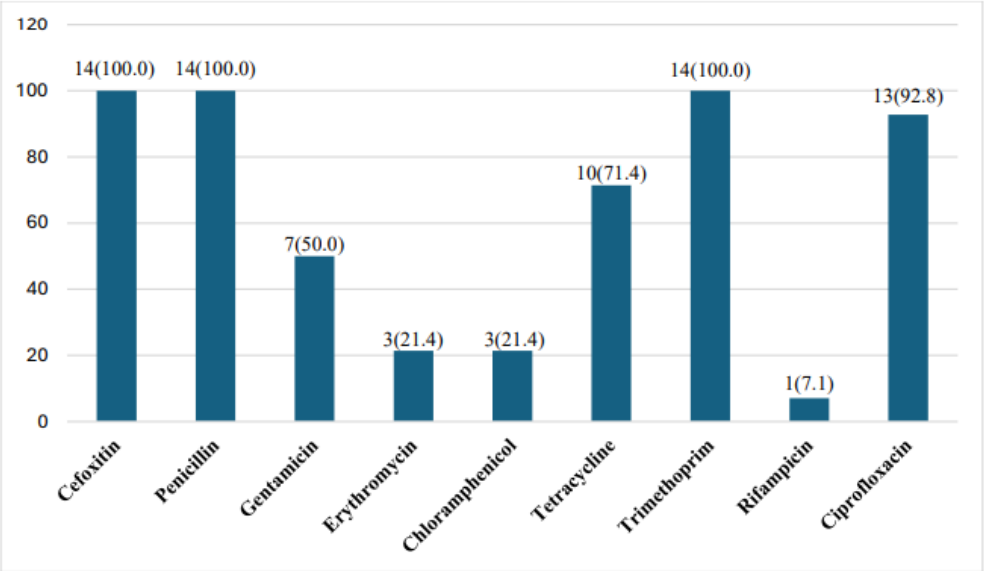

Figure 2: Antibiotic sensitivity profile of MRSA isolates.

CONCLUSION

The study has documented the prevalence of MRSA in *S. aureus* isolated in Federal Medical Center Makurdi, Benue State and provided data on their antibiotic resistance profiles. Also, this study has provided initial data on MRSA obtained in the study area that will serve as a platform for further studies.

ACKNOWLEDGEMENTS

We thank the management of Federal Medical Center Makurdi for giving approval for the study.

Authors Contributions

Conceptualization: Ezra Dasun; Investigation: Ezra Dasun, Unyime C. Essien; Methodology: Unyime C. Essien, Ezra Dasun; Supervision: E. U. Umeh; Writing-original draft: Ezra Dasun; Writing-review and editing: Unyime C. Essien, Davil Nicholas, Nnamdi Harrison Uzoma.

Disclosure of conflict of interest

We have no conflict of interest to disclose.

REFERENCES

1. Abdel-Maksoud, M, El-Shokry, M., Ismail, G., Hafez, S., El-Kholy, A., Attia, E. and Talaat, M., 2016. Methicillin-Resistant *Staphylococcus aureus* Recovered from Healthcare and Community-Associated Infections in Egypt. *International Journal of Bacteriology*, 5751785, 1-5. Doi: org/10.1155/2016/5751785.
2. Abdullahi, N. and Iregbu, K.C. Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. *Annals of Tropical Pathology*, 2018; 9: 6-10. https://www.atpjournal.org/text.asp?2018/9/1/6/234154
3. Adam, K.M. and Abomughaid, M.M. Prevalence of Methicillin-resistant Staphylococcus aureus in Saudi Arabia Revisited: A Meta-analysis. The Open Public Health Journal, 2018; 11: 584-591. Doi:10.2174/1874944501811010584.
4. Adam, K.M. and Abomughaid, M.M. Prevalence of Methicillin-resistant *Staphylococcus aureus* in Saudi Arabia Revisited: A Meta-analysis. *The Open Public Health Journal*., 2018; 11: 584-591. Doi:10.2174/1874944501811010584.
5. Adetayo, T. O., Deji-Agboola, A. M., Popoola, M.Y., Atoyebi, T.J. and Egberongbe, K. J. Prevalence of Methicillin Resistant *Staphylococcus aureus* from Clinical Specimens in Ibadan, Nigeria. *The International Journal of Engineering and Science*, 2014; 3(9): 1-11. https://www.theijes.com.
6. Akanbi, B.O. and Mbe, J.U. Occurrence of methicillin and vancomycin resistant *Staphylococcus aureus* in University of Abuja Teaching Hospital, Abuja, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 2012; 14(1): 18. https://agris.fao.org/agris.
7. Akhter, R., Khan, K.M.A. and Hasan, F. Isolation and antimicrobial susceptibility pattern of methicillin-resistant and methicillin sensitive *Staphylococcus aureus*. *Journal of Surgery Pakistan (International)*, 2009; 14(4): 161-164. Doi:10.11648/j.ajcem.20160401.12
8. ALFouzan W, Boswihi SS, Dhar R, Udo E. Evaluating the antibacterial activity of ceftaroline against clinical isolates of methicillin-susceptible and- resistant *Staphylococcus aureus* in Kuwait hospitals. Journal of Infection Public Health, 2020; (10): 1589-1591. Doi: 10.1016/j.jiph.2020.07.018. Epub 2020 Aug 26. PMID: 32859552.
9. Amoako, D.G., Somboro, A.M., Sekyere, J.O., Moodley, K., Bester, L. and Essack, S.Y.Tet(M) Mediates Tetracycline Resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Clinical Isolates from The Private Hospital Sector in KwaZulu-Natal (KZN), South Africa. *Journal of Pure and Applied Microbiology*, 2019; 13(1): 51-59. Doi: 10.22207/JPAM.13.1.0
10. Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from clinical infection and asymptomatic carriers in Southwest Nigeria. PLoS One, 2015; 10(9): 1-8. Doi:10.1371/journal.pone.0137531.
11. Azeez-Akande, Utsalo, S.J. and Epoke, J. Distribution and Antibiotic Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* Isolates in a University Teaching Hospital in Nigeria. *Sahel Medical Journal*, 2008; 11(4): 142-147. https://www.smjonline.org/article.
12. Bawonda, E.O., Moses, A.E., Etang, U.E. Occurrence of high-level methicillin resistance *Staphylococcus aureus* in patients from health facilities in Akwa Ibom State, Nigeria. Ibom Medical Journal., 2024; 17(1): 56 – 61.
13. Boswihi, S. S., Udo, E. E. and Al-Sweih, N. Shifts in the Clonal Distribution of Methicillin Resistant *Staphylococcus aureus* in Kuwait Hospitals: 1992-2010. *PLoS One*, 2016; 11(9): 1-21. Doi.org/10.1371/journal.pone.0162744
14. Buzaid, N., Elzouki, A., Taher, I. and Ghenghesh, K.S. Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary surgical and trauma hospital in Benghazi, Libya. *Journal* of *Infection in Developing Countries*, 2011; *5*(10): 723-726. Doi.org/10.3855/jidc.1701
15. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Wayne, PA, USA: CLSI; 2017. M100-S24.
16. Darboe, S., Dobreniecki, S., Jarju, S., Jallow, M., Mohammed, N.I., Wathuo, M., Ceesay B., Tweed S., Basu Roy. R., Okomo. U., Kwambana-Adams, B., Antonio, M., Bradbury, R.S., de Silva, T.I., Forrest, K., Roca, A., Lawal, B.J., Nwakanma, D. and Secka, O. Prevalence of Panton-Valentine Leukocidin (PVL) and Antimicrobial Resistance in Community-Acquired Clinical *Staphylococcus aureus* in an Urban Gambian Hospital: A 11-Year Period Retrospective Pilot Study. *Frontiers in Cellular* and *Infection Microbiology*, 2019; *22*(9): 1-9. Doi:org/10.3389/fcimb.2019.00170
17. Deyno, S., Fekadu, S. and Astatkie, A. "Resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia: a meta-analysis," *Antimicrobial Resistance and Infection Control*, 2017; 6(1): 85-91. Doi:10.1186/s13756-017-0243-7
18. Dilnessa, T. and Bitew, A. Antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 hospital medical college, Addis Ababa, Ethiopia. *BioMed Central Infectious Diseases*, 2016; 16(398): 1-9. Doi:10.1186/s12879-016-1742-5
19. Egbuobi, R. C., Wachuku, C. K., Dike-Ndudim J. N., Ogamaka, I. A., Okorie, H. M., Nwagbaraocha, M. A., Amadi, J. C., Egbuobi, L. N. and Ereh, J. E. Evaluation of antibacterial profile of methicillin

resistant Staphylococcus aureus (MRSA) isolated from hospitals in Imo state, Nigeria. International Journal of Medicine and Medical Sciences. 6(2): 69-74. DOI: 10.5897/IJMMS2013.1001.

20. Essien, U.C., Boswihi, S.S., Agbakoba, N.R and Udo, E.E. Molecular typing of Methicillin Resistant *Staphylococcus aureus* obtained from clinical specimens in tertiary hospitals in Jos, Nigeria. World Journal of Advance Healthcare Research, 2021; 5(5): 131-146.
21. Fayyaz, M., Mirza, I.A., Ahmed, Z., Abbasi, S.A., Hussain, A. and Ali, S. In Vitro Susceptibility of Chloramphenicol Against Methicillin-Resistant *Staphylococcus aureus*. *Journal of the College of Physicians and Surgeons Pakistan*, 2013; 23(9): 637-640. https://inis.iaea.org
22. Ghebremedhin B, Olugbosi MO, Raji AM, Layer F, Bakare RA, Konig B, Konig W. Emergence of a Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strain with a Unique Resistance Profile in Southwest Nigeria. Journal of Clinical Microbiology, 2009; 47(9): 2975-2980. Doi:10.1128/JCM.00648-09.
23. Hanci, H., Ayyıldız, A. and Igan, H. Investigation of inducible clindamycin resistance in methicillin resistant *Staphylococcus aureus* strains. *Marmara Pharmaceutical Journal*, 2017; 21(2): 407-411. Doi: 10.12991/marupj.301167
24. Idris AM, Kumurya AS, Mohammed Y, Mustapha HM. Phenotypic determination of methicillin-resistant Staphylococcus aureus in Aminu Kano Teaching Hospital, Kano, Nigeria. *Nigerian Journal of Experimental and Clinical Biosciences*, 2019; 7: 1-6.
25. Ikeh E.I. Methicillin 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
26. Jevons MP. Clebenin-resistant *Staphylococci*. British Medical Journal, 1961; 1(5219): 124-125. https://www.ncbi.nlm.nih.gov.
27. Kaleem, F., Usman, J., Hassan, A., Omair, M., Khalid, A. and Uddin, R. Sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolated from patients admitted in tertiary care hospital of Pakistan. *Iranian Journal of Microbiology*, 2010; 2(3): 143-146. https://www.ncbi.nlm.nih.gov.
28. Khosravi, A.D., Jenabi, A. and Montazeri, E.A. Distribution of genes encoding resistance to aminoglycoside modifying enzymes in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. *Kaohsiung Journal of Medical Sciences*, 2017; 33(12): 587-593. Doi:10.1016/j.kjms.2017.08.001.
29. Kumurya, A.S. Fusidic Acid and Rifampicin Resistant Strains of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Northwestern Nigerian Hospitals. *Agricultural and Biological Sciences Journal*, 2015; 1(4): 132-136. http://www.aiscience.org/journal/absj
30. Lakhundi S, Zhang K. Methicillin resistant *Staphylococcus aureus*: molecular characterization evolution and epidemiology. Clinical Microbiology Reviews, 2018; 31(4): 1-103. Doi: 10.1128/CMR.00020-18.
31. Marais, E., Aithma, N., Perovic, O., Oosthuysen, W.F., Musenge, E. and Duse, A.G. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from South Africa.*South African Medical Journal*, 2009; 99(3): 170-173. http://www.samj.org.za/index.php/samj
32. Mofolorunsho, K. C., Emmanuel, M. T., Omatola C.A., Aminu R. F. and Ocheni H. O. Prevalence and Antibiotic Resistance Profiles of Methicillin-Resistant *Staphylococcus aureus* Isolated from Clinical Specimens in Anyigba, Nigeria. *UMYU Journal of Microbiology Research*, 2022; 7(1): 38-46.
33. Njoungang, L.L., Nwobegahay, J.M., Ayangma, C.R., Njukeng, A.P., Kengne, M., Abeng, E.M., Mama, E.A., Tchouamo, M. and Goon, D.T. Prevalence and antibiotic resistance patterns of strains of *Staphylococcus aureus* isolated at the Yaounde Military Hospital, Cameroon. *Microbiology Research International*, 2015; 3(4): 56-63. http://www.netjournals.org/pdf/MRI/2015/4/15-021.pdf
34. Nsofor CA, Nwokenkwo VN, Ohale CU. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. MedCrave Online Journal of Cell Science and Report, 2016; 3(2): 60-63. Doi:10.15406/mojcsr.2016.03.00054.
35. Nsofor, C.A., Nwokenkwo, V.N.and Ohale, C.U. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. *MedCrave Online Journal of Cell Science and Report*, 2016; 3(2): 60-63. Doi:10.15406/mojcsr.2016.03.00054
36. Nsofor, C.A., Nwokenkwo, V.N. and Ohale, C.U. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. *MedCrave Online Journal of Cell Science and Report*, 2016; 3(2): 60-63. Doi:10.15406/mojcsr.2016.03.00054
37. Nwankwo, E.O.K., Abdulhadi, S., Magagi, A. and Ihesiulor G. Methicillin Resistant *S. aureus* (MRSA) and their Antibiotic Sensitivity Pattern in Kano, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 2010; 11(1): 129-136. Doi:10.4314/ajcem.v11i1.44088.
38. Ogbolu, D., Alli, O., Bello, L. and Ibrahim A. Emergence of vancomycin intermediate Staphylococcus aureus (VISA) in clinical isolates of methicillin resistant *S. aureus* from south western region of Nigeria. *International Journal of Tropical*

Disease and Health, 2015; 10(4): 1-5. Doi:10.9734/IJTDH/2015/20642.
39. Ojulong, J., Mwambu, T.P., Joloba, M., Bwanga, F. and Kaddu-Mulindwa, D. H. Relative prevalence of methicillin resistant *Staphylococcus aureus* and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzania Journal of Health Research*, 2009; 11(3): 149-153. http://www.scholarsresearchlibrary.com
40. Okon, K., Uba, A.B.P., Oyawoye, O.M., Yusuf, I.Z., Shittu, A.O. and Blanc, D.L.J. Epidemiology and characteristic pattern of methicillin-resistant *Staphylococcus aureus* recovered from tertiary hospitals in Northeastern Nigeria. *International Journal of Tropical Medicine*, 2011; 6(5): 106-12. http://www.medwelljournals.com.
41. Okon, K.O., Shittu, A.O., Kudi, A.A., Umar, H., Becker, K. and Schaumburg, F. Population dynamics of *Staphylococcus aureus* from Northeastern Nigeria in 2007 and 2012.*Epidemiology and Infection*, 2014; 142(8): 1737–1740. Doi:10.1017/S0950268813003117.
42. Okon, K.O., Shittu, A.O., Usman, H., Adamu, N., Balogun, S.T. and Adesina, O.O. Epidemiology and Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* Recovered from Tertiary Hospitals in Northeastern, Nigeria. *Journal of Medicine and Medical Sciences*, 2013; 4(5): 214-220. http://www.interesjournals.org/JMMS.
43. Okoye, E. L., Omeje, M. J. ., & Ugwuoji, E. T. Detection and Prevalence of Methicillin and vancomycin resistant Staphylococcus aureus among clinical isolates in ESUTH, Enugu state. *Journal of Current Biomedical Research*, 2022; *2*(2): 170–186. https://doi.org/10.54117/jcbr.v2i2.13.
44. Okwu, M.U., Okorie, T.G., Mitsan, O. and Osakue, E.O. Prevalence and comparison of three methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in tertiary health institutions in Nigeria. *Canadian Open Biological Sciences Journal*, 2014; 1(1): 1-12. Available online at http://crpub.com/Journals.php
45. Olowe, O.A., Eniola, K.I.T., Olowe, R.A. and Olayemi, A.B. Antimicrobial susceptibility and beta-lactamase detection of MRSA in Osogbo, South West, Nigeria. *Nature and Science*, 2012; 5(3): 44-48. http://www.sciencepub.net/nature.
46. Onelum, O., Odetoyin, B., Onipede, A.and Oyelese, A. The Role of Methicillin-Resistant *Staphylococcus aureus* in Clinical infections in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, South Western Nigeria. *Journal of Microbiology and Experimentation*, 2015; 2(2): 59-64. http://medcraveonline.com
47. Pirko, E.Y., Tektook, N.K., Saleh, M.M.S., Jaffar, Z.A. Prevalence of methicillin resistance Staphylococcus aureus (MRSA) and methicillin sensitivity *Staphylococcus aureus* (MSSA) among hospitalized Iraqi patients. *Biomedical Research*, 2019; 30(4): 1-5. http://www.biomedres.info.
48. Rajaduraipandi, K., Mani, K.R. and Panneerselvam K. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicenter study. *Indian Journal of Medical Microbiology*, 2006; 24(1): 34-38. Doi:10.4103/0255-0857.19892.
49. Raji A, Ojemhen O, Umejiburu U, Ogunleye A, Blanc DS, Basset P. High genetic diversity of *Staphylococcus aureus* in a tertiary care hospital in Southwest Nigeria. Diagnostic Microbiology and Infectious Disease, 2013; 77(4): 367-369. Doi.org/10.1016/j.diagmicrobio.2013.08.030
50. Sales, A.J., Farhadi, F., Ezdiyadi, M. and Nazloo, D.T. Study of antibiotic resistance pattern in methicillin-resistant *Staphylococcus aureus* isolated from clinical samples of hospitals in Tabriz-Iran. *International Journal of Biomedicine and Public Health*, 2018; 1(2): 71-75. Doi:10.22631/IJBMPH.2018.116735.1014
51. Shahi, K., Rijal, K.R., Adhikari, N., Shrestha, U.T., Banjara, M.R., Sharma, V.K. and Ghimire, P. Methicillin Resistant *Staphylococcus aureus:* Prevalence and Antibiogram in Various Clinical Specimens at Alka Hospital. *The Tribhuvan University Journal of Microbiology*, 2018; 5(1): 77-82. Doi:10.3126/tujm.v5i0.22316
52. Sit PS, Teh CSJ, Idris N, Sam C, Omar SFS, Sulaiman H, Thong KL, Kamarulzaman A, Ponnampalavanar S. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and the molecular characteristics of MRSA bacteraemia over a two-year period in a tertiary teaching hospital in Malaysia. *BMC Infectious Diseases, 2017; 17(1): 1-14. Doi: 10.1186/s12879-017-2384-y.*
53. Terry Alli, O.A., Ogbolu, D.O., Mustapha, J. and Akinbami, R The non-association of Panton-Valentine leukocidin and *mecA* genes in the genome of *Staphylococcus aureus* from hospitals in South Western Nigeria. *Indian journal of medical microbiology***,** 2012; 30(2): 159-164. Doi:10.4103/0255-0857.96675
54. Udo EE, Farook VS, Mokaddas EM, Jacob LE, Sanyal SC. Molecular fingerprinting of mupirocin-resistant *Staphylococcus aureus* from a burn unit. International Journal of Infectious Diseases, 1999; 3(2): 82-87.
55. Umoh, N. O., Udonkang, M., Akpan, S., Bebia, G., Usanga, V., Igwebuike, N. (2024). Antibiotic Resistance Indices of Methicillin-Resistant Staphylococcus aureus isolates at a Tertiary Healthcare Facility in Calabar, Nigeria. Sokoto Journal of Medical Laboratory Science, 2024; 9(1): 150 – 158.
56. Yilmaz, E.S. and Astantas, O. Antimicrobial resistance and Underlying mechanism in *Staphylococcus aureus* isolates. *Asian Pacific Journal of Tropical Medicine*, 2017; 10(11): 1059-1064. Doi:10.1016/j.apjtm.2017.10.003

57. Yusuf, E. and Airauhi, L. Prevalence and pattern of methicillin resistant *Staphylococcus aureus* in a tertiary healthcare facility in Nigeria. *Medical Journal of Zambia*, 2015; 42(1): 7-11. https://www.ajol.info/index.php.
58. Zhang K, McClure J, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome *mec* Types I to V in Methicillin-Resistant *Staphylococcus aureus*. Journal of Clinical Microbiology, 2005; 43(10): 5026–5033. Doi:10.1128/JCM.43.10.5026-5033.2005.