International Journal of Microbiology and Applied Sciences
Volume 2 issue 2 Sept, 2023
Research Article

Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt

Robinson, V. K*., Wemedo, S. A., Akani, N. P and Amadi, L. O.
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
***Corresponding Author:** victor.robinson3@ust.edu.ng

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant public health concern due to its high resistance to commonly used antibiotics. This study aimed to determine the prevalence of MRSA from clinical specimens collected from a teaching hospital in Port Harcourt and assess its antibiotic susceptibility pattern. A total of 294 specimens which included wound swabs, ear swab, stool, and urine were collected from patients presenting with suspected *Staphylococcus aureus* infections over a 12-month period (May 2022-April, 2023). Specimens were cultured for the presence of *Staphylococcus aureus* on mannitol salt agar. Antibiotic susceptibility pattern and Methicillin-Resistant *S. aureus* (MRSA) were screened using standard laboratory procedures. Of the 294 clinical specimens analyzed, 37 (12.58%) were confirmed to be positive for *Staphylococcus aureus*. Among these, 25 isolates (69.4%) were identified as MRSA. The highest prevalence of MRSA was observed in stool (89%), followed by wound and urine (75%). However, ear specimen showed the lowest MRSA prevalence (38.46%). The MRSA isolates exhibited high resistance rates to commonly prescribed antibiotics, such as cefuroxime, ceftazidime, ceftriaxone, erythromycin, cloxacillin and augmentin, while relatively higher susceptibility was observed to ofloxacin and gentamycin. The prevalence of MRSA in clinical specimens from the teaching hospital in Port Harcourt is substantial, with an alarming level of antibiotic resistance observed against commonly used antibiotics. This highlights the urgent need for antimicrobial stewardship and infection control measures to curb the spread of MRSA in healthcare settings. Prompt identification and appropriate management of MRSA infections are crucial to mitigate its impact on patient outcomes and overall public health.

Keywords: Methicillin-resistant *Staphylococcus aureus*, clinical specimens, urine, ear swab, drug resistance.

Introduction

Antibiotic resistance has become a significant concern in public health sector across the globe. This is because treatment failure orchestrated by resistance frequently has negative effects, especially for patients who are critically ill (Ariom *et al.*, 2019). This also implied that continued resistance of pathogens to antibiotics could lead to increased burden of diseases with no adequate antibiotics for treatment. one of the most important bacterial isolate considered to be a major problem in the health care especially as it has been implicated in nosocomial and community infections is methicillin-resistant *Staphylococcus aureus* (MRSA) (Obianuju *et al.*, 2015). These isolates are known to be resistant to all available beta-lactam antibiotics including other classes of antibiotics (Ghamba *et al.*, 2012).

MRSA causes a wide range of infection such as bacteremia, pneumonia, meningitis, endocarditis, skin and soft tissue infections, surgical site infections, infections of the urinary system, infections of the bones and joints, and toxic shock syndrome (Abubakar and Sulaiman, 2018). According to Ike *et al.*, (2017), MRSA infections are accountable for long hospital stays, soaring medical expenses, and an elevated mortality rate. The range of MRSA infections is wide, and they are all linked to worse outcomes, including increased death, longer hospital stays, and higher treatment costs. The frequency of MRSA varies by region, hospital type, and the population being researched (Obianuju *et al.*, 2015). Methicillin resistance by *S. aureus* has been reported to be mediated by the mecA gene.

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.

Moglad, (2021) in a previous study reported that the staphylococcal cassette chromosome mec (SCCmec) contains the mecA gene, which codes for antibiotic resistance. He further stated that methicillin and other beta-lactam antibiotics have lower affinity for the 78-kDa penicillin-binding protein (PBP2a), which is encoded by the mecA gene. The prevalence of MRSA has been reported to vary by region. In a previous study, the prevalence of non-invasive MRSA was reported to have declined while in Nigeria, available evidence suggested that MRSA infection had become more common.

In 2009, the rate was 18.3%, in 2010, it was 16.5%, and in 2013, it was 42.3% (Abubakar and Sulaiman, 2018). This increased prevalence of MRSA has drawn concerns due to the morbidity and mortality rates the cause. A previous study on the carriage of MRSA in Ile-Ife found that 50% of hospital staff had MRSA on their hands and in their nasal cavities (Onipede *et al.*, 2007). Serious questions are raised regarding the risks to patients and the potential for MRSA to spread outside the healthcare system (Obianuju *et al.*, 2015).

In contrast to the developed world, there is a dearth of recent data on MRSA in patients in Rivers State, Nigeria and available treatment options. In order to determine the prevalence of MRSA in clinical specimens and its antibiotic susceptibility profiles, this study was undertaken.

Materials and Method

Collection of Specimen

The study area was in Rivers State, Nigeria. The study station was the Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria. The study was a cross-sectional study and specimens were collected randomly from patients within the period of May 2022 to April, 2023. Urine and stool specimens were collected in the Microbiology laboratory of the different study area. While ear swab and wound specimen were collected from patients with complains of ear infection and wound infections. Urine and faecal (stool) specimens were collected using standard method of specimen collection on sterile biological specimen bottles (Cheesbrough, 2006).

Isolation of *Staphylococcus aureus*

Swab of ear and wound specimens were cultured as described by previous studies (Motayo *et al.*, 2012; Kassam *et al.*, 2017). On arrival at the Microbiology Laboratory, swab sticks were swabbed onto the surface of freshly prepared sterile mannitol salt and blood agar plates while stool and urine specimens were streaked on the surface of freshly prepared sterile mannitol and blood agar plates (Cheesbrough, 2006). The inoculation was done in duplicates after which the plates were incubated at 37ºC for 24- 48 hours. After incubation, plates were read and those showing golden yellow colonies on mannitol salt agar were subcultured onto nutrient agar plates and incubated at 37ºC for 24 hours.

Preservation and Identification of Isolates

Pure cultures of *Staphylococcus* sp were preserved in bijou bottles containing 3mL sterile glycerol (10%v/v) and were stored frozen in the refrigerator. These pure isolates were used both for the identification process and other tests.

The bacterial isolates were identified using standard method. First, isolates were identified using colonial characters (colour, shape, size, texture and opacity of the colonies), morphological characters (gram stain and motility) and biochemical characters. Biochemical tests adopted include catalase test, growth on blood agar, haemolysis test, oxidase test, Methyl-red test, Voges Proskaeur test, motility test, citrate utilization and sugar fermentation tests (Prescott *et al.*, 2011).

Determination of Antibiotic Susceptibility of Isolates

The antibiotic susceptibility pattern of *Staphylococcus aureus* was carried out according to the Clinical Laboratory Standard Institute (CLSI, 2019). In this method, 24 hours old culture of each test organism were seeded evenly onto the surface of the Mueller-Hinton agar and allowed to dry. Wafers containing the antibiotics were carefully placed on the dried inoculated agar plates and incubated for 24 hours (Robinson *et al.*, 2023; Wemedo and Robinson, 2018).

The diameter of the zone of inhibition after incubation was read and recorded and interpreted according to the CLSI (2019) as resistant, susceptible and intermediate. The gram-positive discs (abtek) used for staphylococcal isolates were Ceftazidime (30µg), Cefuroxime (30µg), Gentamycin (10µg), Ceftriaxone (30 µg), Erythromycin (5µg), Cloxacillin (5µg), Ofloxacin (5µg) and Augmentin (30µg).

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.

Screening for Methicillin Resistant *Staphylococcus aureus* (MRSA)

All the isolates that were positive for *Staphylococcus aureus* were further screened for their methicillin resistant capability. This was done according to the CLSI (2019) guidelines using oxacillin and cefoxitin disc. Staphylococcal isolates from a solution adjusted to 0.5 McFarland standard were inoculated unto Mueller Hinton agar**.** The cefoxitin and oxacillin disc were placed on the already seeded Mueller Hinton agar plates and incubated for 24 hours. Zones of inhibition were interpreted as: ≤ 21mm = resistant and ≥ 22mm = susceptible.

Results

The prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in the specimens is presented in Figure 1. Data showed that 13 (34.21%), 9 (23.68%), 12 (31.58%) and 4 (10.1%) of *S. aureus* was isolated from 60 ear swabs, 73 stool specimen, 88 urine and 73 wound specimens respectively. The prevalence of MRSA in the specimen in ascending order was ear (38.46%) < urine and wound (75%) < stool (89%).

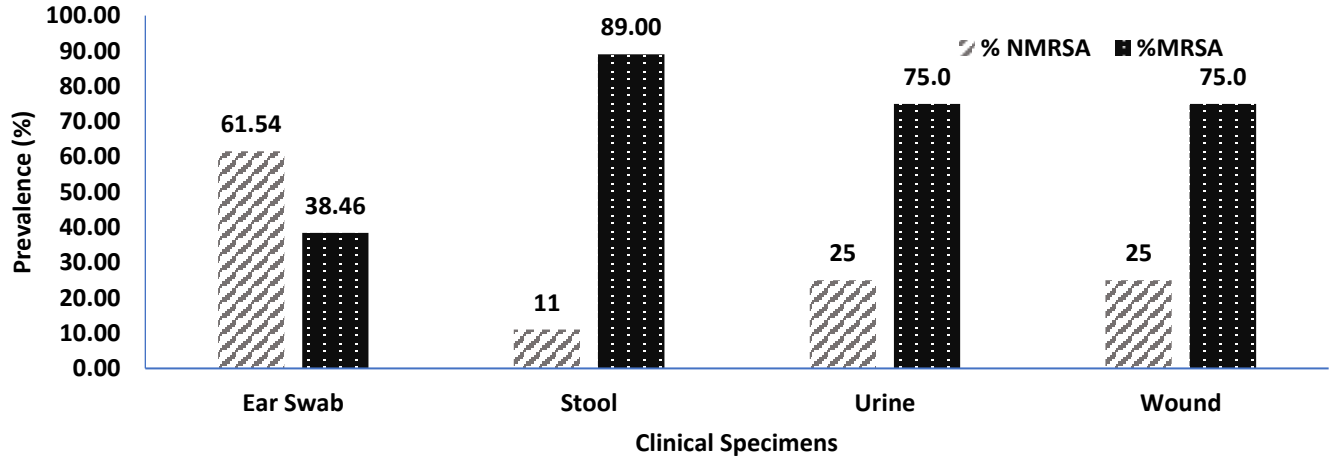

Fig. 1: Prevalence of Methicillin-Resistant *S. aureus* (MRSA) in the specimens

Results of the antibiotics susceptibility pattern of the MRSA isolates from the specimens is presented in Table 1. Data showed that all (100%) of the isolates were resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and augmentin, while 88.9% and 100% were susceptible to gentamycin and ofloxacin, respectively.

Results of the percentage susceptibility of MRSA from stool specimens showed that all (100%) were resistant to ceftazidime, cefuroxime, ceftriaxone, cloxacillin and augmentin, while 87.5%, 87.5% and 12.5% were susceptible to gentamycin, ofloxacin and erythromycin, respectively.

The percentage susceptibility of MRSA isolates from wound specimens showed that 66.7% of the isolates were susceptible to gentamycin and ofloxacin respectively, while all (100%) were resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and augmentin. Similarly, the susceptibility pattern of MRSA isolated from ear specimens showed that only 80 and 100% of the isolates were susceptible to gentamycin and ofloxacin, respectively, while all (100%) the isolates were resistant to ceftazidime, cefuroxime, ceftriaxone, cloxacillin, erythromycin and Augmentin.

The result of the MAR index is presented in Table 2. Results showed that 3 (37.5%) of the isolates from stool specimens had MAR index of 0.3, while 5 (62.5%) had MAR index of 0.6. About 8 (88.9%), 2(66.7) and 4 (80%) of the stool, wound and ear isolates had MAR index of 0.8.

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.

Table 1: Antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimen

Antibiotics	Urine, n = 9		Stool, n = 8		Wound, n = 3		Ear, n = 5	
	R [n (%)]	S [n (%)]	R [n (%)]	S [n (%)]	R [n (%)]	S [n (%)]	R [n (%)]	S [n (%)]
Ceftazidime (30µg)	9 (100)	0	8 (100)	0	3 (100)	0	5 (100)	0
Cefuroxime (30 µg)	9 (100)	0	8 (100)	0	3 (100)	0	5 (100)	0
Gentamycin (10 µg)	1 (11.1)	8 (88.9)	1 (12.5)	7 (87.5)	1 (33.3)	2 (66.7)	1 (20)	4 (80)
Ceftriaxone (30 µg)	9 (100)	0	8 (100)	0	3 (100)	0	5 (100)	0
Erythromycin (5 µg)	9 (100)	0	7 (87.5)	1 (12.5)	3 (100)	0	5 (100)	0
Cloxacillin (5 µg)	9 (100)	0	8 (100)	0	3 (100)	0	5 (100)	0
Ofloxacin (5 µg)	0	9 (100)	1 (12.5)	7 (87.5)	1 (33.3)	2 (66.7)	0	5 (100)
Augmentin (30 µg)	9 (100)	0	8 (100)	0	3 (100)	0	5 (100)	0

Keys: S – susceptible, R – Resistant, n – number of isolates

Table 2: MAR indices and percentage resistance of the *S. aureus* isolates from clinical specimens

MAR Index	Clinical specimens			
	Urine, n = 9(%)	**Stool, n = 8 (%)**	**Wound, n = 3(%)**	**Ear, n = 5(%)**
0.1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.3	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)
0.6	0 (0.0)	5 (62.5)	0 (0.0)	0 (0.0)
0.7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.8	8 (88.9)	0 (0.0)	2 (66.67)	4 (80)
1.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used

Discussion

The prevalence of *Staphylococcus aureus* isolated from the stool specimens was higher than those isolated from ear, urine and wound specimens. High prevalence of *Staphylococcus aureus* in urine specimens have been reported in previous study. Ghamba *et al*. (2012) in their study of reoccurrence and distribution of methicillin- resistant *S. aureus* in clinical specimens in Bauchi, Nigeria reported that *S. aureus* was more prevalent in urine specimens. The prevalence rate (45.3%) of *S. aureus* reported in their study was higher than the 36.1% and 33.3% isolated from ear and urine specimens in the present study. Similarly, Gaire *et al.* (2021) reported a higher prevalence of 41.8% *S. aureus* in urine specimen which was higher than *S. aureus* isolated from other specimen. The present study contradicts previous study which reported low prevalent rate of 2.8% of *S. aureus* in urine specimens (Dilnessa and Bitew, 2016).

Despite the high *S. aureus* isolated in the present study, the findings showed that the number of *S. aureus* isolated from the specimens was very low. This was reflected in the total number of specimen (294) of which only 37 (12.25%) showed growth of *S. aureus.* Low growth rate of bacterial isolates from clinical specimens suspected to be infected have been reported in previous study (Gaire *et al.*, 2021) which agreed with the present study.

The prevalence of MRSA in the present study is higher than the 28.0% recorded by Ghamba *et al.* (2012) in North eastern Nigeria and the 17.7% to 25% reported in Ebonyi State, Nigeria (Ariom *et al.*, 2019). More so, the prevalence of MRSA in the present study is higher than the prevalence of 26.9% of *S. aureus* reported by Ibrahim *et al*. (2020) and is beyond the range determined in a previous report of Gorwitz *et al*. (2015) which put the prevalence in Nigeria at the range of 21%–30%.

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.

Although Onemu and Ophori (2013) had reported a high prevalent rate of 79% in Benin which is higher than the present study.

The highest MRSA isolates was recorded in urine specimen while the wound specimen had the least MRSA in specimen. This finding does not agree with previous studies which had reported higher prevalence of MRSA in wound specimens even though they all considered urine as the second most prevalent specimen for MRSA (Obianuju *et al.,* 2015; Ibrahim *et al*., 2020). The disparity in the MRSA across the specimen could be attributed to difference in specimens as well as factors affecting the *S. aureus* in the specimen or the characteristics of the strains of *Staphylococcus aureus* (Moglad, 2021).

The isolates exhibited multi-drug resistance especially as they resisted more than 3 antibiotics and had MAR indices greater than 0.2. MAR index greater than 0.2 have been reported to indicate that the isolates originated from a high risk source of contamination where antibiotics are often used and possibly abused (Udobi *et al.,* 2013). This implied that areas or environments with frequent use of a particular antibiotics could habour antibiotics resistant genes than areas with less antibiotics use or environments lacking antibiotics resistant genes. Thus, this could be the case in the present study which showed that most of the isolates had MAR indices >0.2. Resistance to gentamycin and ofloxacin were also recorded even though susceptibility to gentamycin and ofloxacin varied across the respective specimen types. As earlier stated, resistance to antimicrobial agents could be influenced by many factors including the geographic regions (Moglad, 2021). Susceptibility of *S. aureus* isolates to gentamycin and ofloxacin have been reported in previous studies. This finding is consistent with (Nwankwo *et al.,* 2009) who reported susceptibility of MRSA to gentamycin and ofloxacin. More so, susceptibility of the isolates of urine specimen to gentamycin was lower than the susceptibility recorded in ofloxacin. Thus, ofloxacin showed higher susceptibility in this specimen compared to the gentamycin antibiotics while in the stool specimen, gentamycin and ofloxacin have same level of sensitivity against the isolates. In wound and ear specimen, the isolates were more susceptible to ofloxacin than gentamycin.

High susceptibility rate to gentamycin have been reported in previous study and it was opined that this high rate of effectivity could be attributed to the drug being in injection form which is not readily abused as those on tablets (Ike *et al.,* 2017). More so, gentamycin as an aminoglycoside is known to inhibit protein synthesis in bacteria by binding to the 30S ribosomal subunit of the bacterial cells while ofloxacin which is a quinolone is known to interfere or inhibit nucleic acid synthesis (Prescott *et al.,* 2011). High susceptibility of MRSA to gentamycin have been reported in previous studies (Ariom *et al*., 2019; Obianuju *et al*., 2015). The characteristic MDR feature of MRSA was well observed in this study with respect to ceftriaxone, ofloxacin, erythromycin, gentamicin, and cefuroxime. The presence of insertion sites for plasmids and transposons in mecA complex of MRSA which often carry antibiotics resistance genes could account for the resistance to several classes of antibiotics (Ibrahim *et al.,* 2020).

In conclusion, the present study has demonstrated high prevalence of multi-drug resistant MRSA in clinical specimens. The presence of these resistant isolates could be a major health risk in spreading antibiotics resistant genes, especially if these specimens are discarded into the environment without sterilization. Furthermore, the high resistance rate of antibiotics highlights the need for judicious use of antibiotics in order to limit the emergence of MRSA resistance.

References

Abubakar, U., and Sulaiman, S. A. S. (2018). Prevalence, trend and antimicrobial susceptibility of Methicillin Resistant *Staphylococcus aureus* in Nigeria: a systematic review. *Journal of Infection and Public Health*. *11(6)*: *763–770*.

Ariom, T. O., Iroha, I. R., Moses, I. B., Iroha, C. S., Ude, U. I., and Kalu, A. C. (2019). Detection and phenotypic characterization of methicillin-resistant Staphylococcus aureus from clinical and community samples in Abakaliki, Ebonyi State, Nigeria. *African Health Sciences*. *19(2)*: *2026–2035*.

Dilnessa, T., and Bitew, A. (2016). 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*. *16(1)*: *1–9.*

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.

Gaire, U., Thapa Shrestha, U., Adhikari, S., Adhikari, N., Bastola, A., Rijal, K. R., Ghimire, P., and Banjara, M. R. (2021). Antibiotic Susceptibility, Biofilm Production, and Detection of mecA Gene among *Staphylococcus aureus* Isolates from Different Clinical Specimens. *Diseases*. *9(4): 80*.

Ghamba, P. E., Mangoro, Z. M., and Wasa, D. E. (2012). Reoccurrence and distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical specimens in Bauchi , North eastern Nigeria. *Journal of Medicine and Medical Sciences*. *3*: *506–511*.

Ibrahim, K. O. O., Adepoju, G. F., Owoeye, J. F. A., Abdulmajeed, A. A., and Folaranmi, O. O. (2020). Orbital Mesenchymal Chondrosarcoma : Report of a Rare Tumor in a Nigerian Girl. *Annals of Tropical Pathology*. *11(2)*: *20–23*.

Ike, B., Ugwu, M. C., Ikegbunam, M. N., Nwobodo, D., Ejikeugwu, C., Gugu, T., and Esimone, C. O. (2017). Prevalence, Antibiogram and Molecular Characterization of Comunity-Acquired Methicillin-Resistant *Staphylococcus aureus* in AWKA, Anambra Nigeria. *The Open Microbiology Journal*. *10(1)*: *211–221*.

Moglad, E. H. (2021). Prevalence of Methicillin-Resistant Staphylococcus aureus (Mrsa) in Clinical Specimens and Among Hospital Staff Nasal Carriers in Khartoum State. *International Journal of Pharmaceutical Sciences and Research*. *12(1)*: *673–677*.

Nwankwo, B., Abdulhadi, S., Magagi, A., and Ihesiulor, G. (2009). Methicillin resistant S. aureus (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. *African Journal of Clinical and Experimental Microbiology*. *11(1)*: *29–33*.

Obianuju, O., Babatunde, O., Anthony, O., and Adesola, O. (2015). 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*. *2(2)*: *59–64*.

Onipede, A. O., Ijadunola, K. T. and Faponle, A. F. (2007). A preliminary report of methicillin-resistant *Staphylococcus aureus* carriage among hospital staff in critical care units in Ile-Ife. *Nigerian Journal of Health sciences. 8(1): 30-34*.

Robinson, V. K., Aleruchi, O., Okafor, A. C., Ahuokpo, H. I., and Ipalibo, C. H. (2023). Antibiogram and Resistant Gene (Gentamicin and Estended Beta-lactam) Profile of *Escherichia coli* Isolated from Yoghurt. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. *14(2)*: 1–11.

Udobi, C. E., Obajuluwa, A. F., and Onaolapo, J. A. (2013). Prevalence and antibiotic resistance pattern of methicillin-resistant staphylococcus aureus from an orthopaedic hospital in nigeria. *BioMed Research International*. *2013*: *1–4*.

Wemedo, S. A., and Robinson, V. K. (2018). *Evaluation of Indoor Air for Bacteria Organisms and their Antimicrobial Susceptibility Profiles in a Government Health Institution*. *11(3)*: *1–7*.

Citation: Robinson *et al*. (2023). Prevalence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Clinical Specimens from a Teaching Hospital in Port Harcourt. *International Journal of Microbiology and Applied Sciences*. *2(2)*: 31 - 36.