***Original Research Article***

# Multi-drug resistance pattern of *Staphylococcus aureus* from Paediatric ward, General Hospital, Ikot-Ekpaw, Mkpat Enin LGA, Akwa-Ibom State, Nigeria.


## ABSTRACT

**AIM:** This study evaluated the Multi-drug resistance (MDR) pattern of *Staphylococcus aureus* from a paediatric ward and was conducted using standard clinical microbiological procedures.

**LOCATION AND DURATION OF STUDY**: The study was carried out on infant samples collected from the Paediatric ward, General Hospital, Ikot-Ekpaw, Mkpat Enin LGA, Akwa-Ibom State, Nigeria, over three (3) months period.

**DESIGN OF STUDY:** Hundred swab-samples were inoculated on Mannitol salt agar. Positive growths were further biochemically confirmed for *Staphylococcus aureus.* Confirmed isolates were then used for MDR evaluation.

**RESULT AND INTERPRETATION:** Of the 100 samples from skin, wound, ear, throat and nose swabs, 28 isolates were confirmed as *S. aureus* and were subjected to a range of selected commercially available antibiotics like: amoxicillin, ampiclox, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, levofloxacin, norfloxacin, rifampicin and streptomycin, to evaluate their susceptibilities. The wound swabs gave the highest isolate percentage yield (32%) followed by skin swabs (29%). Susceptibility results showed that amoxicillin and ampiclox were more resisted by the isolates, while ciprofloxacin, levofloxacin and norfloxacin were more effective against the isolates. The Multiple antibiotics resistance (MAR) indices showed that 85.7% of the isolates had confirmed multi-drug resistance status, with 60.7% of the isolates showing resistance to between four or more of the tested antimicrobials. MAR indices revealed that 96.4% of the isolates had 0.3, indicating that the resistance resulted from isolates that adapted to the tested drugs due to some form of abuse. Restricted use of these drugs would help curtail the high resistance currently observed amongst microorganisms.



Key words: multi-drug resistance, *Staphylococcus aureus,* multiple antibiotics resistance index, susceptibility test, nosocomial infections


## INTRODUCTION

Microbes do manifest themselves in three ways, through substance spoilage, fermentation of organic and inorganic matters and causation of ailments. Different microorganisms, with their different mode of aetiology, causing different types of ailments, will require different methods

and effective drugs for treatments. Continuous deployment of antimicrobial drugs in treating microbial infections has led to the emergence of resistance amongst various strains of microorganisms [1, 2]. Multi-drug resistance (MDR) literally means 'being resistant to more than one antimicrobial agent', although a standardized definition has not yet been agreed upon by the medical community. There are currently other definitions that are used to characterize multidrug resistance. The most practical definition used for Gram-positive and Gram-negative bacteria is 'resistance to three or more antimicrobial classes according to Magiorakos *et al*. [3]. MDR could also be defined as the insensitivity or resistance of a microorganism to administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to the same medicines [4].

Comment [ACI1]: Patterns deleted.

According to Nikaido [5], multidrug resistance in bacteria cells come about by their accumulation of resistance (R) plasmids or transposons, or genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug type. This MDR abilities lead to ineffective ailment treatment, resulting in its persistence, infections spread and high cost [2, 6].

The hospital environment has been said to be an active reservoir for infectious microorganisms, being the meeting point for people with diverse disease etiological agents and susceptible individuals [7, 8, 9]. Nikaido [5] mentioned that *Staphylococcus aureus* has a known nosocomial, multi-drug-resistant strain referred to as the methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA was initially controlled but currently is also resistant to other antimicrobials like the aminoglycosides, chloramphenicol, lincosamides, macrolides and tetracycline [1]. This study was conducted as part of evidence to buttress the efficacy of Staphylococcal infections in young children and the scale to which MDR pathogens are becoming threats to the health of the younger generation amongst the Mkpat Enin, Akwa-Ibom State populace.

Comment [ACI2]: Italics removed. Staphylococcal is not a name of an organism.

## MATERIALS AND METHODS

### Study Facility, Group and Sample Collection

The study facility is in a growing town and services a couple of adjoining communities, with a number of established institutions, stable commerce and ever growing population. The General

Hospital, Ikot-Ekpaw, in Mkpat Enin LGA, Akwa-Ibom State, South-South Nigeria, has Out-patient department, Post-natal ward, Paediatric ward and servicing laboratories. The study focused on children aged 1-15 years.

One hundred (100) samples from skin, nostrils, wound, throat and ear were collected over a period of three months using sterile swabs. Once gotten, the swabs were labelled, placed in an ice pack and taken to the Microbiology laboratory, Akwa-Ibom State University.

Comment [ACI3]: There is nothing like sterile swab samples.

**Sample Analysis**

The samples were dipped into peptone water and incubated at 37$^{o}$C for 24 hours. A loopful from each sample was streaked on separate Mannitol salt agar (MSA) plates and incubated at 37$^{o}$C for 24 hours. Discrete golden yellow colonies were subcultured, purified and preserved. Only Gram positive cocci bacterial colonies were further tested for catalase and coagulase.

**Antibiotics Susceptibility Test**

Confirmed *Staphylococcus aureus* isolates were tested for their susceptibilities to various selected commercial antibiotic drugs like Ciprofloxacin, Erythromycin, Levofloxacin, Gentamicin, Ampiclox, Rifampicin, Amoxicillin, Streptomycin, Norfloxacin and Chloramphenicol. Overnight cultures using Kirby-Bauer method [10] were inoculated on Mueller-Hinton agar (Oxoid, Uk), cultures adjusted to 0.5 McFarland standard. After pre-diffusion, the plates were incubated at 37$^{o}$C for 24 hours. Diameter of zones of inhibition (IZDs) produced by the antibiotics were measured and recorded in millimeter. Thereafter, the Multiple antibiotics resistance (MAR) index was determined for each isolate using the formula MAR = *x/y,* where *x* is the number of antibiotics to which test isolate displayed resistance and *y* is the total number of antibiotics to which the test organism has been evaluated for sensitivity [11, 12].

## RESULTS AND DISCUSSIONS

Result for the prevalence of *S. aureus* is as shown in Table 1. The result table shows that of the 28 samples that yielded *S. aureus*, wound samples had the highest number, which was followed by samples from children skin swabs. The least number of confirmed *Staphylococcus aureus* isolates were from the ear swabs.

The susceptibility pattern of the 28 confirmed test isolates to the selected commercially available drugs (amoxicillin, ampiclox, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, levofloxacin, norfloxacin, rifampicin and streptomycin) is as shown in Figure 1. Ciprofloxacin

was the most effective drugs against the test organism, followed by Levofloxacin and Norfloxacin. Confirmed *Staphylococcus aureus* isolates had high resistance for Amoxicillin, closely followed by their resistance for Ampiclox.

**Table 1: Prevalence of *Staphylococcus aureus* from clinical samples**

| Type of Specimen | Sample Size | Number of *S. aureus* isolated | Total percentage |
|---|---|---|---|
| Skin | 20 | 8 | 29 |
| Nose | 20 | 5 | 18 |
| Wound | 20 | 9 | 32 |
| Throat | 20 | 4 | 14 |
| Ear | 20 | 2 | 7 |

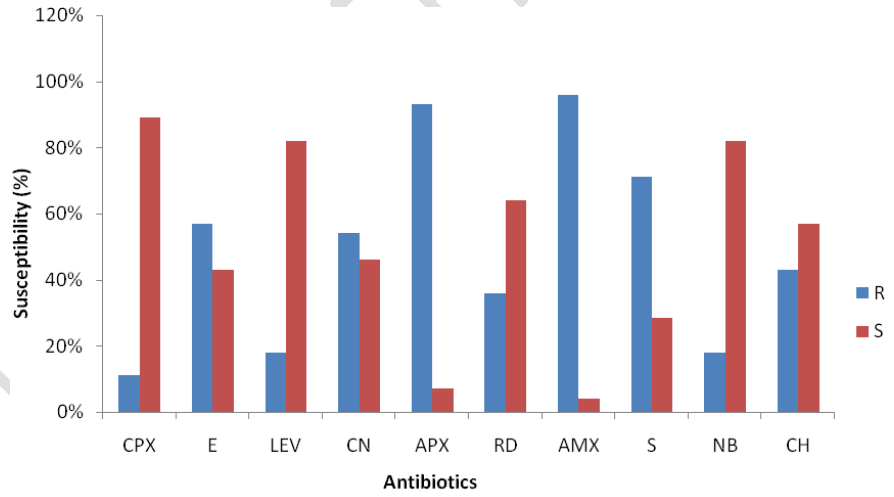


**Figure 1: Antibiotics susceptibility pattern of *Staphylococcus* strains from clinical sample**

**KEY:** R= Resistant, S= Sensitive, CPX= Ciprofloxacin, E= Erythromycin, LEV= Levofloxacin, CN= Gentamicin, APX= Ampiclox, RD= Rifampicin, AMX= Amoxicillin, S= Streptomycin, NB= Norfloxacin, CH= Chloramphenicol

Table 2 has the multiple antibiotics resistance (MAR) index result which shows that 85.7% of the confirmed test isolates were multi-drug resistant (showing resistance to three or more classes of antibiotics). Only 14.3% of the isolates showed resistance to only two classes of antibiotics. Results from this study show that 60.7% of test isolates had resistance for four or more antibiotics drugs. MAR index value for 96.4% of the test isolates reveal cases of source drug abuse.

**Table 2: Antibiotic Resistance Pattern and MAR index of *Staphylococcus aureus***

| S/N | ISOLATE CODE | Antibiotic Resistant Pattern | MARI | Antibiotic Resistant Class |
|---|---|---|---|---|
| 1 | S9 | RD, S | 0.2 | RIF, AMG |
| 2 | S8 | APX, AMX, CH | 0.3 | PEN, CHL |
| 3 | N3 | LEV, APX, AMX | 0.3 | QUI, PEN |
| 4 | W1 | CN, AMX, CH | 0.3 | AMG, PEN, CHL |
| 5 | W9 | APX, AMX, S | 0.3 | PEN, AMG |
| 6 | S10 | CN, APX, RD, AMX | 0.4 | AMG, PEN, RIF |
| 7 | T6 | E, APX,AMX, NB | 0.4 | MAC, PEN, QUI |
| 8 | T8 | CPX, E, APX, AMX | 0.4 | QUI, MAC, PEN |
| 9 | S2 | E, APX, RD, AMX, S | 0.5 | MAC, PEN, RIF, AMG |
| 10 | S3 | E, CN, APX, AMX, S | 0.5 | MAC, AMG, PEN |
| 11 | N1 | E, CN, APX, AMX, CH | 0.5 | MAC, AMG, PEN, CHL |
| 12 | N2 | CN, APX, AMX, S, CH | 0.5 | AMG, PEN, CHL |
| 13 | W2 | E, LEV, APX, AMX, S | 0.5 | MAC, QUI, PEN, AMG |
| 14 | W5 | E, APX, AMX, S, CH | 0.5 | MAC, PEN, AMG, CHL |
| 15 | W6 | CN, APX, AMX, S, CH | 0.5 | AMG, PEN, CHL |
| 16 | W7 | APX, AMX, S, NB, CH | 0.5 | PEN, AMG, QUI, CHL |
| 17 | W10 | E, LEV, APX, AMX, S | 0.5 | MAC, QUI, PEN, AMG |
| 18 | T3 | E, APX, AMX, S, CH | 0.5 | MAC, PEN, AMG, CHL |
| 19 | E5 | E, CN, APX, RD, AMX | 0.5 | MAC, AMG, PEN, RIF |
| 20 | S1 | E, CN, APX, RD, AMX, S | 0.6 | MAC, AMG, PEN, RIF |
| 21 | S5 | E, LEV, APX, RD, AMX, S | 0.6 | MAC, QUI, PEN, RIF,AMG |
| 22 | S6 | CN, APX, RD, AMX, S, CH | 0.6 | AMG, PEN, RIF, CHL |
| 23 | N5 | CPX, CN, APX, AMX, S, CH | 0.6 | QUI, AMG, PEN, CHL |
| 24 | W8 | E, CN, APX, AMX, S, NB | 0.6 | MAC, AMG, PEN, QUI |
| 25 | N8 | E, CN, APX, RD, AMX, S, NB | 0.7 | MAC, AMG, PEN, RIF, QUI |

| | | | | |
|---|---|---|---|---|
| 26 | W3 | LEV, CN, APX, RD, AMX, S, NB | 0.7 | QUI, AMG, PEN, RIF |
| 27 | T2 | E, CN, APX, RD, AMX, S, CH | 0.7 | MAC, AMG, PEN, RIF, CHL |
| 28 | E9 | CPX, E, CN, APX, AMX, S, CH | 0.7 | QUI, MAC, AMG, PEN,CHL |

**KEY:** CPX= Ciprofloxacin, E= Erythromycin, LEV= Levofloxacin, CN= Gentamicin, APX= Ampiclox, RD= Rifampicin, AMX= Amoxicillin, S= Streptomycin, NB= Norfloxacin, CH= Chloramphenicol, MARI= Multiple antibiotic resistance index, RIF= Rifamycins, AMG= Aminoglycosides, PEN= Penicillins, CHL= Chloramphenicol, MAC= Macrolides, QUI= Quinolones

Data for isolate occurrence and confirmation showed that there were more confirmed *Staphylococcus* isolates from wound swab-samples, followed by skin swab-samples. Parta *et al.* [13] also recorded very high *Staphylococcus* number from wound swabs. This high isolate-numericals is suggestive of the exposed nature of the sampling points. This is supported by findings presented by Nimmo *et al.* [14], who found more *Staphylococcus* isolates on exposed body surfaces than the internal parts. While uncovered wounds have sticky surfaces and the skin is continuously exposed, it is therefore easy for such high microbial numbers to be recorded.

Susceptibility data showed that all the confirmed and tested isolates resisted two or more antimicrobials. Qureshi *et al.* [15] also isolated MRSA that resisted multiple anti-microbials from hospital specimens. This study's result showed a higher MDR percentage than the "nearly half" proportion reported by Nimmo *et al.* [14].

More than 96.4% of the MAR indices were 0.3 from this study evaluation. This assertion is indicative that resistance to these multiple drugs come from over exposure of the isolates to drugs, making them adapt or resistant to them with recurrent treatments. The high case of MDR amongst microorganisms can drastically be reduced by mere restricting the indiscriminate and readily availability of these drugs over the counter [14].

## CONCLUSION

In conclusion, many studies have shown that prolonged stays in hospitals increases the risk for the colonization or infection with MDR *Staphylococcus aureus.* This reflects an inherent risk in acquiring MDR organisms through environmental contaminations and hospital stay conditions. With infant patients, another potential transmission route is through infected staff members handling and this calls for special care [16, 17].

The isolation of MDR *Staphylococcus aureus* from infant patients is a call for the proper implementation of contact precautions during hospitalization especially in health facilities across developing countries [16, 18].

**Ethical consideration**

The study was approved by the ministry of health, Akwa Ibom State. Permission was obtained from General Administration of the General hospital prior to collecting any data. Participants' privacy and confidentiality have been assured (no names have been used, only numbers were used) and all data and results have been handled and treated confidentially.

**REFERENCES**


1 McIntosh, J. (2018). Antibiotic resistance: What you need to know. Medical news today, (https://www.medicalnewstoday.com/articles/283963.php).

2 Tanwar, J., Das, S., Zeeshan, F. and Hameed, S. (2014). Multidrug Resistance: An Emerging Crisis. *Interdisciplinary Perspectives on Infectious Diseases*, 7 pages.

3 Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, l. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber J. T. and Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, 18, 268–281.

4 Singh, V. (2013). Antimicrobial resistance*In:Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*, FormatexResearchCenter, 1, 291-296.

5 Nikaido, H. (2009). Multidrug Resistance in Bacteria. *Annual Review of Biochemistry*, 78, 119–146.

6 WHO (2014). *Antimicrobial Resistance Global Report on Surveillance*. Geneva, Switzerland: World Health Organization.

7 Zhanel, G. G., DeCorby, M., Laing, N., Weshnoweski, B., Vashisht, R., Tailor, F., Nichol, K. A., Wierzbowski, A., Baudry, P. J., Karlowsky, J. A., Lagace´-Wiens, P., Walkty, A., McCracken, M., Mulvey, M. R. and Johnson, J. (2008). The Canadian Antimicrobial Resistance Alliance (CARA), and Hoban DJ (2008). Antimicrobial-resistant pathogens in intensive care units in Canada: results of the Canadian National Intensive Care Unit (CAN-ICU) study, 2005-2006. *Antimicrobial Agents and Chemotherapy*, 52, 1430-1437.

8 Rhomberg, P. R., Fristoche, T. R., Sader, H. S. and Jones, R. N. (2006). Antimicrobial susceptibility pattern comparisons among intensive care unit and general ward Gram negative isolates from meropenem yearly susceptibility test Information programme (USA). *Diagnostic Microbial Infectious Disease*, 56, 57-62.

9 O'Brien, F. G., Pearman, J. W., Gracey, M., Riley, T. V. and Grubb, W. B. (1999). Community strain of methicillin-resistant S*taphylococcus aureus* involved in a hospital outbreak. *Journal of Clinical Microbiology*, 37(9):2858–2862.

10 Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology,* 23 pages.

11 Tula-Sanchez, A. A., Havas, A. P., Alonge, P. J., Klein, M. E., Doctor, S. R., Pinkston, W., Glinsmann-Gibson, B. J., Rimsza, L. M. and Smith, C. (2013). A model of sensitivity and resistance to histone deacetylase inhibitors in diffuse large B cell lymphoma: Role of cyclin-dependent kinase inhibitors. *Cancer Biology and Therapy*, 14*(*10): 949-961.

12 Olayinka, A. T., Onile, B, A. and Olayinka, B. O. (2004). Prevalence of multi-drug resistance (MDR) *Pseudomonas aeruginosa* isolates in surgical units of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria: An indication for effective control measures. *Annals of African Medicine,* 3(1): 13-16.

13 Parta, M., Goebel, M., Matloobi, M., Stager, C. and Musher, D. M. (2009). Identification of Methicillin-Resistant or Methicillin-Susceptible*Staphylococcus aureus*in Blood Cultures andWound Swabs by GeneXpert. *Journal of Clinical Microbiology*, 47(5):1609–1610.

14 Nimmo, G. R., Pearson, J. C., Collignon, P. J., Christiansen, K. J., Coombs, G. W., Bell, J. M., McLaws M. L. and the Australian Group on Antimicrobial Resistancem (2011). Antimicrobial susceptibility of *Staphylococcus aureus* isolated from hospital in-patient. *Community DiseaseIntelligence*, 35(3):237–243.

15 Qureshi, A. H., Rafi, S., Qureshi, S. M. and Ali, A. M. (2004).The Current Susceptibility Patterns of Methicillin Resistant *Staphylococcusaureus* to Conventional Anti-Staphylococcus Antimicrobials at Rawalpindi. *Pakistan Journal of Medical Science,* 20(4): 361-364.

16 Buke, C., Armand-Lefevre, L., Lolom, I., Guerinot, W., Deblangy, C., Ruimy, R., Andremont, A. and Lucet J. C. (2007). Epidemiology of multidrug-resistant bacteria in patients with long hospital stays. *Infection Control and Hospital Epidemiology,* 28(11):1255–1260.

17 Maamar, E., Ferjani, S., Jendoubi, A., Hammami, S., Hamzaoui, Z., Mayonnove-Coulange, L., Saidani, M., Kammoun, A., Rehaiem, A., Ghedira, S., Houissa, M., Boutiba-Ben Boubaker, I., Slim, A. and Dubois, V. (2016). High prevalence of gut microbiota colonization with broad-spectrum cephalosporin resistant enterobacteriaceae in a tunisian intensive care unit. *Frontiers in Microbiology,* 7, 1859.

18 Harbarth, S., Sax, H., Fankhauser-Rodriguez, C., Schrenzel, J., Agostinho, A. and Pittet, D. (2006). Evaluating the probability of previously unknown carriage of MRSA at hospital admission. *American Journal of Medicine* 119(3):275.e15-e23