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 - Make in format as aim, location and duration of study, design of study, result and interpretation; leave the space before mentioning of units

The evaluation of the Multi-drug resistance pattern of *Staphylococcus aureus* from a paediatric ward, in Akwa-Ibom State was conducted using standard clinical microbiological procedures. 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%). While 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 MAR indices showed that 85.7% of the isolates had confirmed multi-drug resistance status, with 60.7% of the isolates having resistance for between four or more 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 experienced amongst microorganisms.

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

INTRODUCTION – sequentially number the references mentioned in this section in square bracket

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 capable drugs for treatments. Continuous deployment of antimicrobial drugs in treating microbial infections has led to the emergence of resistance amongst various strains of microorganisms (McIntosh, 2018; Tanwar *et al.,* 2014). 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 patterns 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*. (2012). 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 (Singh, 2013).

According to Nikaido (2009), multidrug resistance in bacteria cells come about by their accumulation, on resistance (R) plasmids or transposons, of 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, infection's spread and high cost (Tanwar *et al.,* 2014; WHO, 2014).

The hospital environment have been said to be a active reservoir for infectious microorganisms, being the meeting point for people with diverse disease etiological agents and susceptible individuals (Zhanel *et al*., 2008; Rhomberg *et al*., 2006; O'Brien *et al.,* 1999). Nikaido (2009) 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 (McIntosh, 2018). 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.

MATERIALS AND METHODS - sequentially number the references mentioned in this section in square bracket after introduction; leave the space before mentioning of the units

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.

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

Sample Analysis

The sterile swab sticks were depth 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 (Hudzicki, 2009) were inoculated on Mueller-Hinton agar (Oxoid, Uk), cultures adjusted to 0.5 McFarland standard. After pre-diffusion, the plates were inoculated 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 determine for each isolate using a 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 (Tula-Sanchez*et al.*, 2013; Olayinka *et al.*, 2004).

RESULTS – combine discussion with this, leave the space before mentioning of the units

Result for the prevalence of *S. aureus* is as shown in Table 1. The result table shows that of the 28 confirmed, 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

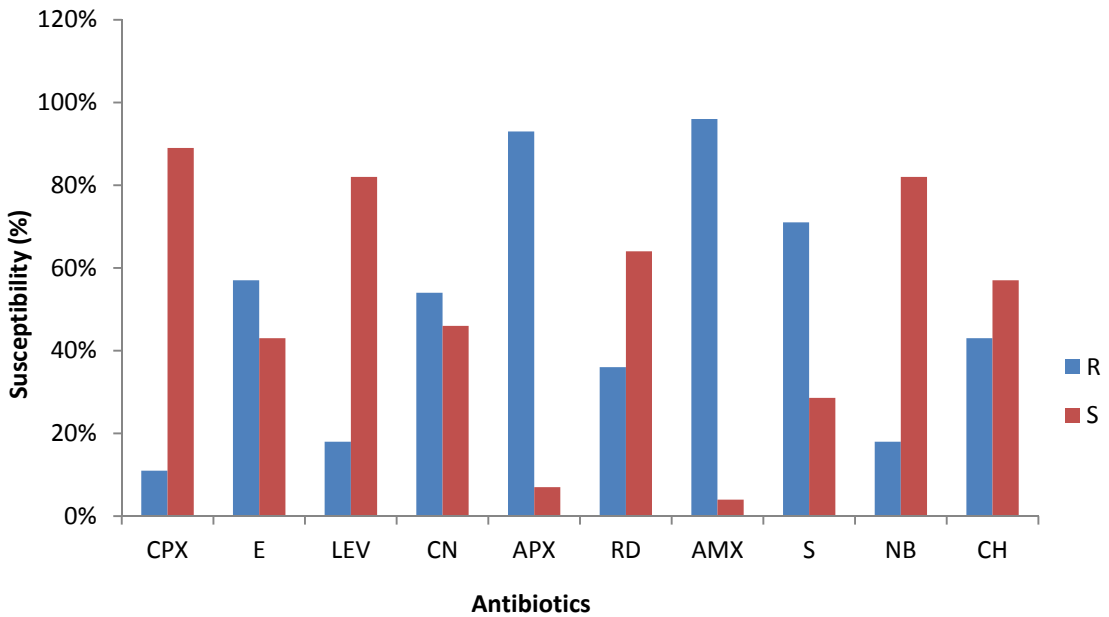

Figure 1: Antibiotics susceptibility pattern of *Staphylococcus* strains from clinical sample

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

DISCUSSIONS AND CONCLUSION - combine discussion with result section, leave the space before mentioning of the units; separate conclusion; sequentially number the references mentioned in this section in square bracket continuously

Data for isolate occurrence and confirmation showed that there were more confirmed *staphylococcus* isolates from wound swab-samples, followed by skin sample-swabs. Parta *et al.* (2009) 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.* (2009), 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.* (2004) 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.* (2009).

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 (Nimmo *et al.*, 2009).

Many studies have shown that prolonged stays in hospitals increases the risk for 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 (Buke *et al*., 2007; Maamar *et al*, 2016).

The isolation of MDR *Staphylococcus aureus* from infant patients is a call for the proper implementation of contact precautions during hospitalization especially in developing countries (Harbarth *et al.*, 2006; Buke *et al.*, 2007).

Ethical consideration

The study was approved by the ministry of health, Akwa Ibom State **(Give the reference no.).** 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 – Continuously mention the references from introduction, method, discussion

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.

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

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

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.

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.

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

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

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.

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.

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.

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.

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.

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.

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

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

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.

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

Zhannel, G. G., Corby, D., Laing, N., Weshionweski, B., Vashisht, R. and Tailer, F. (2008). Canadian Antimicrobial Resistant Pathogens in Intensive care Unit (CAN-ICU) study 2005-2006. *Antimicrobial Agents and Chemotherapy*, 52, 1430-1437.