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Antibiotics Resistance and Plasmid Curing Studies of *Staphylococcus aureus* associated with Wound Infection amongst Patients Accessing University of Port Harcourt Teaching Hospital, Rivers State, Nigeria

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Abstract

Staphylococcus aureus is a common hospital and community-acquired pathogen known to be frequently associated with wound infections, and has become important for its increasing level of resistance to antibiotics. Resistance of *Staphylococcus aureus* to antibiotics could be as a result of the presence of plasmids or resistance genes. The need to carry out plasmid curing of the isolates is very essential. The aim of the study was therefore to determine the antibiotics resistance pattern of *staphylococcus aureus* associated with wound infection amongst patients accessing university of Port Harcourt Teaching Hospital, Rivers State, Nigeria. A total of 150 specimens from different types of wounds (traumatic wound, caesarean section, scrotal wound, surgical wounds, burns, diabetic foot, and plastic surgery) were collected from the Hospital for a period of six months and processed for isolation of *S. aureus*, following standard microbiological procedures. The specimens were cultured on sterile Mannitol Salt Agar (MSA) plates and incubated at 37ºC for 24 hours. Antibiotic susceptibility tests of the isolates was performed using the Kirby-Bauer disc diffusion method, following CLSI guidelines. Plasmid genes of the isolates were cured using acridine orange. The study showed a decrease in antibiotic resistance of 17.3% for ofloxacin (OFL), 13.8% for gentamicin (GEN) and 3.5% for both ceftazidime (CAZ) and ceftriatone (CTR) after plasmid curing, with no apparent change in resistance for the rest of the antibiotics. The results of this study represent serious public health concerns, thus emphasizing the need for proper wound management.

Keywords: Antibiotic resistance; Plasmid curing; Patients; *Staphylococcus aureus;* Wound infection.

1. Introduction

Wound generally refers any damage to the integrity of biological tissue, including skin, mucous membranes, and organ tissues. A wound considered as a type of injury which happens relatively quickly in which the skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the epidermis of the skin [1, 2]. Wound infection on the other hand occurs due to a dynamic interaction between the host and the pathogen [3]. Would contamination could be by pathogens from the external environments or by the normal flora of the host. Infection can occur on acute wounds such as surgical wounds (surgical site infections) and on chronic wounds such as pressure ulcers, diabetic foot ulcer and leg ulcers, which are more likely to be colonized with bacteria due to the nature of the open wound and tissue type [4]. The pathogens most commonly associated with wound infections are *Staphylococcus aureus*, *Streptococcus* species, *Pseudomonas aeruginosa* and anaerobes [5].

Researchers [6-8] have implicated *Staphylococcus aureus* as a frequent wound contaminant. Also, an earlier report by Sampson, *et al.* [9] observed an overall prevalence of 38.7% in the wound cases studied, with the majority of *Staphylococcus aureus* obtained from burn injuries. Pathogenicity of *Staphylococcus aureus* is mainly related to the repertoire virulence factors including toxins, invasive capacity, adhesins, exoenzymes, and immune-modulating proteins it produces, as well as a variety of resistance mechanisms to many existing antibiotics. In a precious study, biofilm formation, hemolysis where evaluated as virulence factors that facilitated the pathogenic potentials *Staphylococcus aureus* due wound colonization. This present study however focused on antibiotics resistance as a factor affecting wound management in clinical settings.

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity, with most targeting bacterial functions or growth processes [10]. Bacterial resistance to conventional antibiotics have been widely reported in recent studies [11-16]. *Staphylococcus aureus* is however, one of the most

significant pathogens in terms of antibiotic resistance, as it has been able to develop resistance mechanisms to nearly all antibiotics used against it [17]. Infections by some species of staphylococci are difficult to treat because of frequency of multiple antibiotic resistant strains. The genes for antibiotic resistance in *Staphylococcus aureus* may be located on plasmids and may confer resistance to a number of antimicrobial agents [18]. Studies carried out to determine the role of plasmids in antibiotic resistance has been useful in determining the characteristics of plasmids in bacteria. Plasmids can be transferred from one close bacterium to another horizontally, while for bacterium that is distant from one another plasmids can be transferred phylogenetically [19]. These two modes of transfer of plasmids might be responsible for the spread of antibiotic resistance genes in the environment [20].

The increasing level of resistance of *Staphylococcus aureus* to antibiotics could be as a result of the presence of plasmids or resistance genes. Therefore the need to carry out plasmid curing of *Staphylococcus aureus* in wound is very essential. The aim of the study was therefore, to determine the antibiotics resistance profile of *Staphylococcus aureus* associated with wound infection amongst patients accessing university of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

2. Materials and Methods

2.1. Description of Study Location

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), East West Road Port Harcourt, Rivers State, Nigeria. The hospital lies within 4.8998^{o} North and 6.9292^{o} East. It is a major tertiary healthcare and research facility in Rivers State, which consists of various departments for distinct health cases, and a great number of patients from many geographical regions accessing it.

2.2. Study Design and Sample Collection

A cross-sectional (prevalence) study design was implemented to determine the incidence of *Staphylococcus aureus* associated with wound infection at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. The study sampled one hundred and fifty (150) wound cases collected randomly from patients with different wound types in the hospital using sterile swab sticks. The samples were taken aseptically and transported to the Microbiology Laboratory, Rivers State University.

2.3. Sample Size Determination

The sample size for the study was determined by the formula [20]:

$N = [Z^2(pq)]/d^2$ [20]

Where:

N= the desired sample size

Z= Normal standard distribution that corresponds to confidence interval as 1.96

p= Prevalence of *Pseudomonas* species

q = 1-p

d= degree of accuracy / precision expected at 0.05

2.4. Isolation of Staphylococcus Aureus

The different swab sticks specimens were plated on sterile Mannitol Salt Agar (MSA) plates using the streak plate method and incubated at 37ºC for 24 hours.

2.5. Purification and Preservation of Isolates

The distinct tentative *Staphylococcus* colonies on the MSA plates were further purified on freshly prepared Nutrient Agar (NA) plates by repeated subculturing until pure colonies (isolates) were obtained as described by Sampson, *et al.* [9]. Obtained pure isolates were inoculated aseptically into nutrient agar slants in Bijou bottles and incubated for 24 hours at 37ºC. After incubation, agar slants were then refrigerated at 4^{o}C to preserve the isolates.

2.6. Preparation of 0.5M Mcfarland Turbidity Standard

About 1% v/v solution of Sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and properly mixed. About 0.5g of dehydrated Barium Chloride ($Bacl_2$.$2H_2O$) was dissolved in 50ml of distilled water to prepare 1% w/v of Barium Chloride Solution [21]. About 0.6ml of the Barium Chloride solution was added to 99.4ml of the sulphuric acid solution and properly mixed. A prepared turbid solution was transferred to a capped tube and kept in well-sealed container in the dark at room temperature (25-28ºC).

2.7. Antibiotic Susceptibility Test by Disc Diffusion Method

A sterile swab stick was dipped into the tube containing the bacterial suspension and its turbidity was equivalent to 0.5m McFarland turbidity. The swab stick was pressed against the tube above the fluid level to remove excess broth. The swab was used to streak over the entire plate surface evenly which contained already prepared Mueller-Hinton agar in three dimensions rotating the plate about 60^{o}C each time. The agar plate was allowed to dry for 5 minutes then the antibiotic disk was impregnated to the agar using a sterile forcep on the surface of the inoculated plate 15mm away from the edge of the plate. Using the head of the sterile forcep the disk is slightly pressed down to ensure good contact with the agar. After applying the disk, the plates were inq1cubated in an inverted position at

35^{o}C for 16 to 18 hours. After incubation the test plates were examined to ensure confluence growth or near confluence. The diameter of each zone of inhibition was measured in ml using a ruler on the underside of the plate and recorded for reference purpose [21].

2.8. Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance is the resistance of isolates of *Staphylococcus aureus* to three or more antibiotics [15]. Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula, MAR = a/b; where a denoted the number of antibiotics to which the test isolate depicted resistance and b for the total number of antibiotics to which the test isolate has been evaluated for susceptibility.

2.9. Plasmid Curing

This procedure was used to screen for and eliminate plasmids present in the staphylococcal isolates which could be responsible for antibiotic resistance. Luria Bertani (LB) broth was used as medium and acridine orange (0.5 mg/ml) was the curing agent. To carry out this procedure, a loopful of each isolate was aseptically inoculated into LB tubes containing the acridine orange and incubated at 37^{0}C for 24hrs. The tubes were agitated at intervals to maintain an even distribution of the curing agent. After incubation, cured isolates were further subjected to antibiotic resistance screening to detect for plasmid-borne antibiotic resistant species.

3. Results

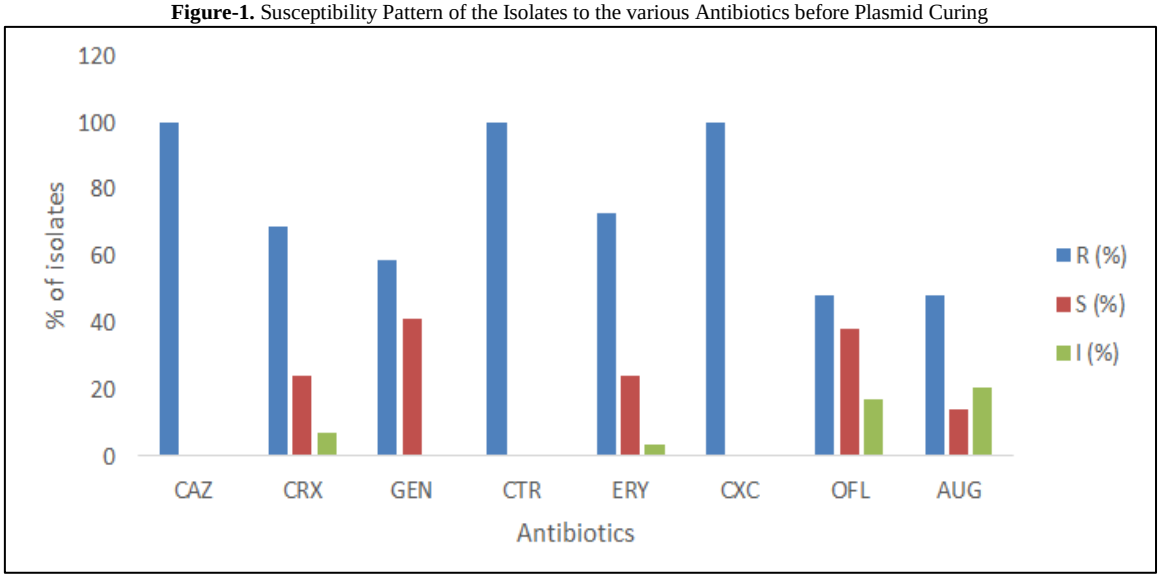
The susceptibility pattern of the isolates to the various antibiotics before plasmid curing showed that all the isolates (100%) were resistant to Ceftazidime, Ceftriaxone and Cloxacillin, as indicated in Figure 1. This was followed by erythromycin (ERY), 72.8%; cefuroxime (CRX), 68.9%; gentamicin (GEN), 58.6%; ofloxacin (OFL), 48.3% and augmentin (AUG), 48.2%.

The overall percentage reduction in antibiotic resistance of *Staphylococcus aureus* after plasmid curing is as shown in Figure 2. The data revealed that Ofloxacin showed the highest percentage reduction (17%) in antibiotics resistance following the treatment with acredine orange, with no reduction observed for Cefuroxime, Erythromycin and Augmentin.

The average MAR index of the isolates before and after plasmid curing is reported in Figure 3. The data indicated a reduction (albeit at varying levels) in MAR index of all the isolates except for those associated with burns, diabetic foot ulcer and plastic surgery, that witnessed no reduction.

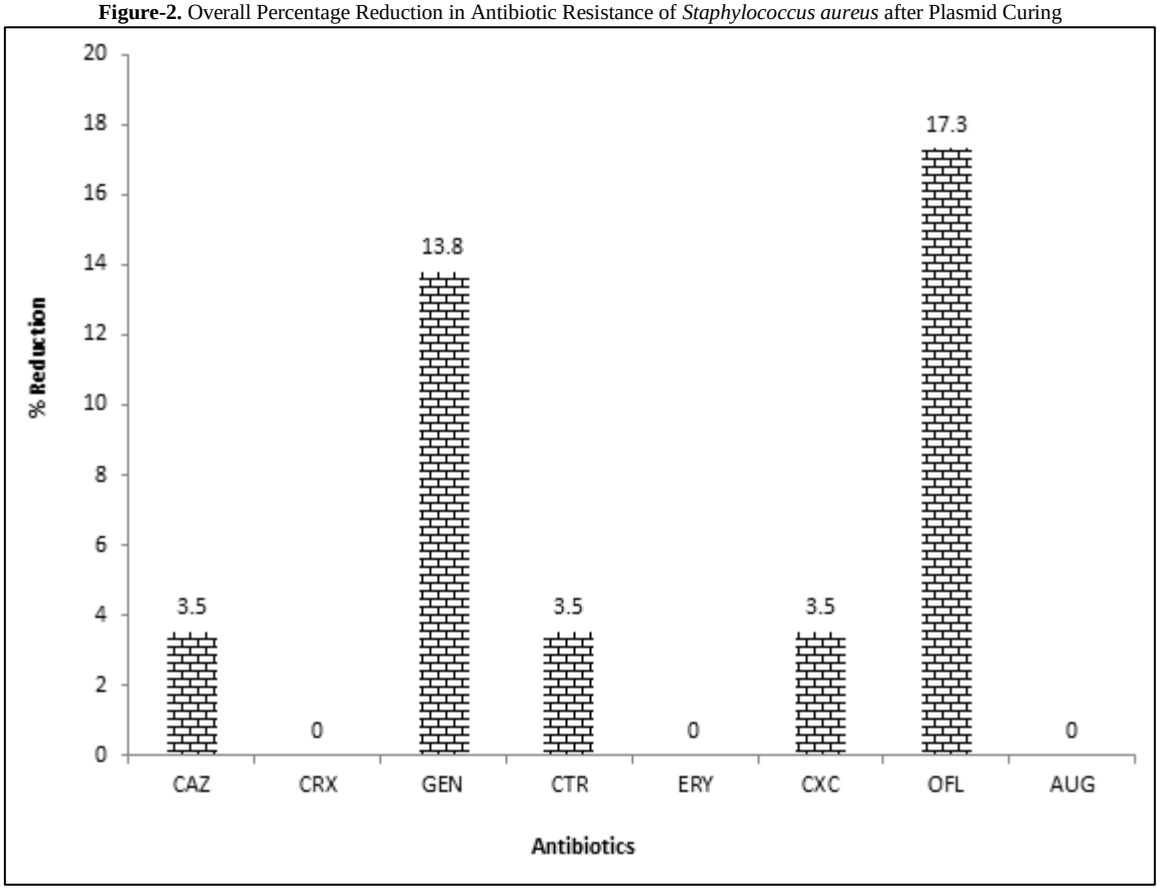
As shown in Figure 3, prior to curing, average MAR index for Ceasarean section was 0.9 for ScrW, 3.33 for S/W, 0.9 for SgW, 1 for A/E, 0.7 for Burns, 0.6 for DFU, 0.5 and P/S, 1. After curing however, MAR index for Ceasarean section wound became 0.775; ScrW, 0.725; S/W, 0.6; SgW, 0.8; A/E, 0.63; B, 0.6; DFU, and 1 for P/S. Percentage reduction in MAR index after plasmid curing (Figure 4) indicated that most reduction happened in Traumatic wound (33.33%), followed by C/S (13.88%), A/E (10%) and ScrW (3.33%) wound types. The other wound types (SgW, B, DFU and P/S) had no change in their average MAR index.

Figure-1. Susceptibility Pattern of the Isolates to the various Antibiotics before Plasmid Curing



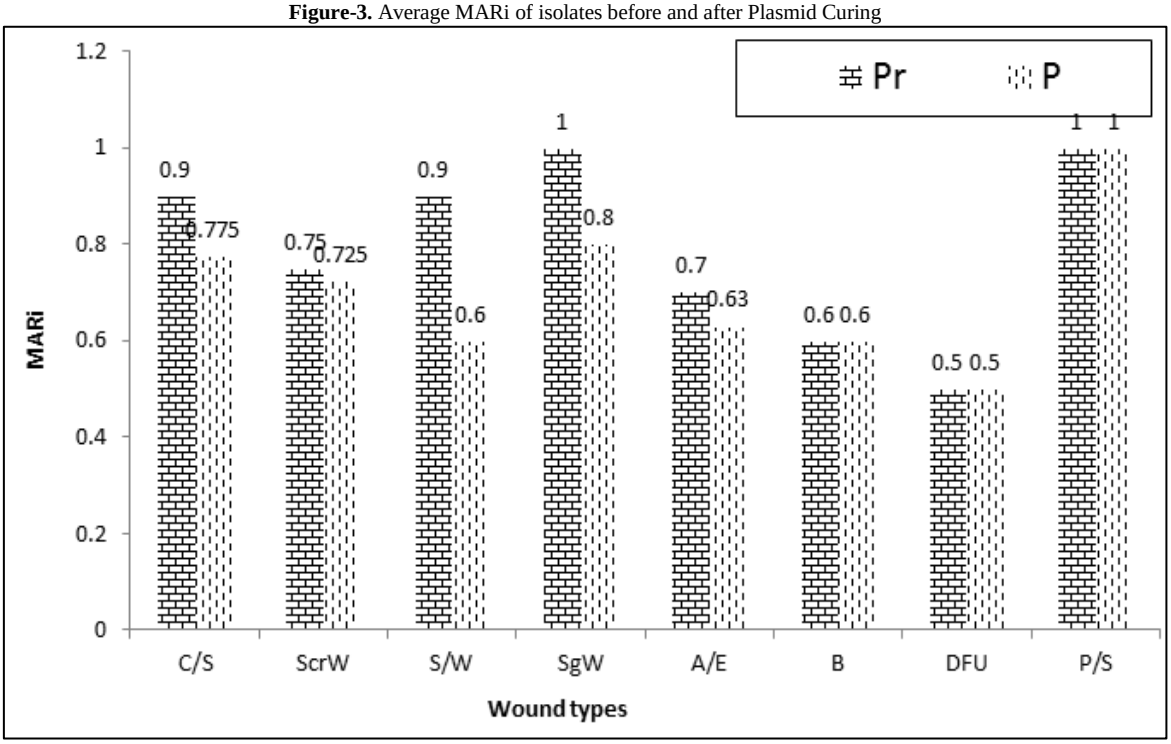
Key:
CAZ = Ceftazidime); **CRX** = Cefuroxime; **GEN** = Gentamicim; **CTR** = Ceftriaxone; **ERY** = Erythromycin; **CXC** = Cloxacillin; **OFL** = Ofloxacin; **AUG** = Augmentin

Figure-2. Overall Percentage Reduction in Antibiotic Resistance of *Staphylococcus aureus* after Plasmid Curing



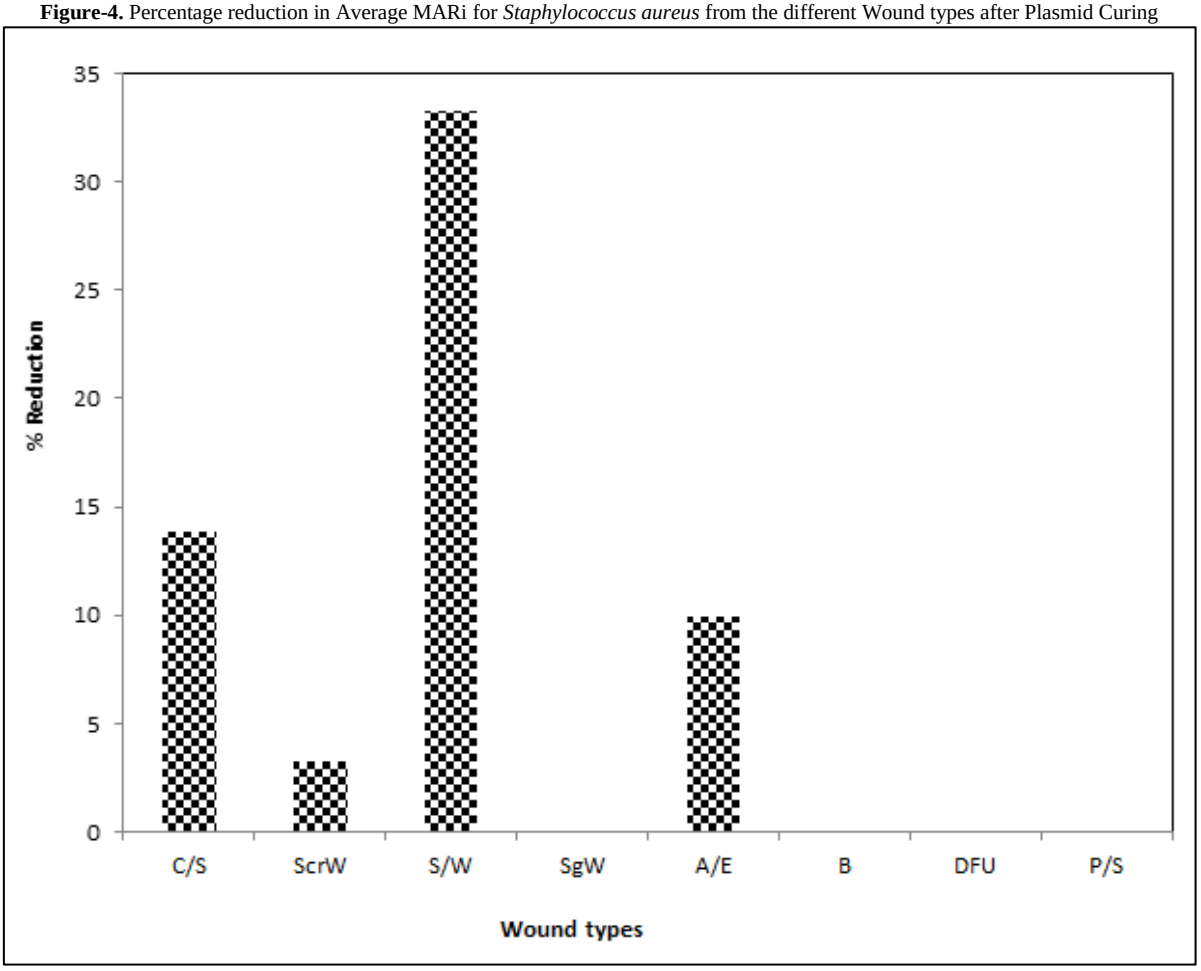
Key:
CAZ = Ceftazidime); **CRX** = Cefuroxime; **GEN** = Gentamicim; **CTR** = Ceftriaxone; **ERY** = Erythromycin; **CXC** = Cloxacillin; **OFL** = Ofloxacin; **AUG** = Augmentin

Figure-3. Average MARi of isolates before and after Plasmid Curing



Key:
C/S = cesarean section; **ScrW** = scrotal wound; **S/W** = surface wound; **SgW** = surgical wound; **A/E** = accident and emergency; **B** = burns; **DFU** = diabetic foot ulcer; **P/S** = plastic surgery; **Pr:** Pre plasmid curing; **P:** Post plasmid curing.

Figure-4. Percentage reduction in Average MARi for *Staphylococcus aureus* from the different Wound types after Plasmid Curing



Key:
C/S = cesarean section; **ScrW** = scrotal wound; **S/W** = surface wound; **SgW** = surgical wound; **A/E** = accident and emergency; **B** = burns; **DFU** = diabetic foot ulcer; **P/S** = plastic surgery

4. Discussion

The study aimed at evaluating the antibiotics resistance pattern of *Staphylococcus aureus* associated with wound infection amongst patients accessing University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. Since *Staphylococcus aureus* is a normal skin flora, it was important to assess different types of wounds to decipher the pattern *of* staphylococcal wound contamination and the associated pattern of antibiotic resistance in clinical settings.

The susceptibility profile observed in this study indicated the isolates showed different pattern of susceptibility to conventional antibiotics. While all the all the isolates were resistant to Ceftazidime, Ceftriaxone and Cloxacillin, the isolates were resistant to the other antibiotics in different proportion.

Several factors have been known to account for bacterial resistance to antibiotics, including plasmid mediated factors as well as some chromosomal factors. The pattern of antibiotics susceptibility of the isolates reported in this study in similar to previous studies by other researchers [17, 18].

A good number of traits are encoded by plasmids (extra chromosomal pieces within the bacterial cytoplasm), which are expressed by the bacterial species. Genes for virulence factors, antibiotic resistance, detoxifying agents and enzymes for secondary metabolism have been found to be associated with plasmids [22].

Antibiotics resistant genes play an important role in drug resistance of an isolate. These genes may be chromosomal or plasmid mediated. From the study, the overall percentage reduction in antibiotic resistance of *Staphylococcus aureus* after plasmid curing was determined. The data indicated that Ofloxacin showed the highest percentage reduction in antibiotics resistance following the treatment with acredine orange, with no reduction observed for Cefuroxime, Erythromycin and Augmentin. This observation is in line with the reports of earlier researchers [23-25]. The notable reduction in antibiotic resistance of the species to these antibiotics could therefore be attributed to the presence of resistance plasmid genes, which were eliminated after plasmid curing. The proportion not reversed after the acridine orange treatment may be an indication of chromosomal contributions [26].

MAR index is an indication of the level of exposure of a given organism to different antibiotics, as it is an index to measure antibiotic resistance level. An index of ≥ 0.2 is an indication of resistance to more than one drug, and increasing values relates with the number of drugs the isolate is resistant to Finberg, *et al.* [27]. As presented in results before and after curing, average MAR index for the different wound types ranged from 0.2 to 1.0. This is a clear indication that the different wounds were consistently exposed to different classes of antibiotics during the treatment course thereby bringing about the high indices in MAR. High MAR indices have been reported to contribute to the development of superbugs [28]. It is therefore in the best interest to prevent such by running

necessary test on every isolated pathogen to determine a specific class of antimicrobial that is effective on it, so as to rule out the use of multiple class of antimicrobials that may facilitate the onset of multidrug resistance.

5. Conclusion

With regards to the data generated from the entire course of study, it can be inferred that the Staphylococcal isolates from the contaminated wound samples associated with multiple antibiotics resistance. This will therefore constitute therapeutic fails in would management and eventual prolong hospitalization and frequent hospital visits.

The persistently high average MAR index value brings us to the conclusion that the organism may have been previously exposed to different classes of antibiotics in the cause of administering treatment to the wounds in the hospital, and as such can account for their high resistance pattern different antibiotic classes. The post plasmid curing sensitivity pattern of the isolates further indicates that while some isolates resistant due to the possession of drug resistant plasmid, other may have been resistant due to chromosomal factors.

Consent and Ethical Approval

As per international standard guideline participant consent and ethical approval has been collected and preserved by the authors.

Competing Interests

Authors have declared that no competing interests exist.

References

[1] Kujath, P. and Michelsen, A., 2008. "Wounds - from physiology to wound dressing." *Deutsches Arzteblatt International*, vol. 105, pp. 239-248.

[2] Wilkins, R. G. and Unverdorben, M., 2013. "Wound cleaning and wound healing: a concise review." *Advances in Skin Wound and Care*, vol. 26, pp. 160-163.

[3] White, R. J., 2009. "Wound infection-associated pain." *Journal of Wound Care*, vol. 18, pp. 245–249.

[4] Vazquez-Boland, J., Stachowiak, R., Lacharme, L., and Scortti, M., 2006. *Listeriolys. In: Alouf, j. E. And popoff, m. R. (eds.). The comprehensive source book of bacterial protein toxins*. Amsterdam: Elsevier Academic Press. pp. 700–716.

[5] Cooper, R., 2005. *Understanding wound infection. In: Cutting, k., gilchrist, b. And gottrup, f. (eds.). Identifying criteria for wound infection. European wound management association position document*. London: MEP Ltd., p. 43.

[6] Frank-Peterside, N. and Sampson, T., 2009. "Pattern of bacterial contamination of wounds at federal medical centre, yenegoa, bayelsa state, Nigeria." *Asian Journal of Microbiology, Biotechnology and Environmental Science*, vol. 11, pp. 501-505.

[7] Wangoye, K., Mwesigye, J., Tungotyo, M., and Samba, S. T., 2022. "Chronic wound isolates and their minimum inhibitory concentrations against third generation cephalosporins at a tertiary hospital in Uganda." *Scientific Reports*, vol. 12, p. 1195.

[8] Lipsky, B. A., Berendt, A. R., and Cornia, P. B., 2012. "Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections." *Clinical Infectious Disease*, vol. 54, pp. e132–e173.

[9] Sampson, T., Alexander, J., and Ugboma, C. J., 2022. "Incidence of Staphylococcus aureus wound infection amongst patients attending University of Port Harcourt Teaching Hospital, Rivers State, Nigeria." *Saudi Journal of Pathology and Microbiology*, vol. 7, pp. 307-312.

[10] Calderon, C. B. and Sabundayo, B. P., 2007. *Antimicrobial classifications: Drugs for bugs. In schwalbe, r., steele-moore, l. And goodwin, a. C. (eds.). Antimicrobial susceptibility testing protocols. Crc press. Taylor and frances group. 45cooper, r. (2005). Understanding wound infection. In: Cutting, k., gilchrist, b. And gottrup, f. (eds.). Identifying criteria for wound infection. European wound management association position document*. London: MEP Ltd., p. 43.

[11] Akani, N. P., Hakam, I. O., and Sampson, T., 2019. "Prevalence and antibiogram of pseudomonas aeruginosa isolated from west african mud creeper (tympanotonus fuscatus)." *South Asian Journal of Research in Microbiology*, vol. 5, pp. 1-8.

[12] Mah, T. F., 2012. "Biofilm-specific antibiotic resistance." *Futurse Microbiol*, pp. 1061-72.

[13] Sampson, T., Akani, N. P., and Aniwoko, O., 2021. "Molecular characterisation and antibiogram of vaginal flora from students attending a tertiary institution in port harcourt, rivers state, Nigeria." *International Journal of Pathogen Research*, vol. 7, pp. 17-27.

[14] Sampson, T., Akani, N. P., and Hakam, I. O., 2020. "Molecular characterization and detection of antibiotic resistance genes in pseudomonas species isolated from tympanotonus fuscatus." *Journal of Advances in Microbiology*, vol. 20, pp. 37-45.

[15] Sampson, T., Barika, P. N., Peekate, L. P., and Akani, N. P., 2020. "Prevalence and antibiogram of escherichia coli isolated from edible cockle (senilia senilis) in rivers state, Nigeria." *International Research Journal of Public and Environmental Health*, vol. 7, pp. 149-156.

[16] Sampson, T. and Emelogu, G. O., 2022. "Prevalence and antibiogram of biofilm forming bacteria associated with toilet seats in dormitories within a university campus in Port Harcourt, Rivers State, Nigeria." *South Asian Journal of Research in Microbiology*, vol. 13, pp. 43-51.

[17] Foster, T. J., 2017. "Antibiotic resistance in staphylococcus aureus. Current status and future prospects." *FEMS Microbiology Reviews*, vol. 7, pp. 430–449.

[18] Simpson, A. H., Dave, J., and Cookson, B., 2007. "The value of routine screening of staff for MRSA." *Journal of Bone and Joint Surgery British*, vol. 89, pp. 565-566.

[19] Dale, J. W. and Park, S., 2004. *Molecular genetics of bacteria*. 4th ed. Chichester, UK.: John Wiley and Sons Inc., pp. 22-24.

[20] Niang, L., Winn, T., and Nordin, R., 2006. "Practical issues in calculating the sample size for prevalence studies." *Archives of Orofacial Sciences*, p. 1

[21] Clinical and Laboratory Standards Institute, 2017. "Performance standards for antimicrobial susceptibility testing, Approved Standard M100-S25."

[22] Smillie, C., Garcillán-Barcia, M. P., Francia, M. V., Rocha, E. P., and de la Cruz, F., 2010. "Mobility of plasmids." *Microbiology and Molecular Biology Reviews*, vol. 74, pp. 434–452.

[23] Akinjogunla, O. J. and Enabulele, I. O., 2010. "Virulence factors, plasmid profiling and curing analysis of multi-drug resistant Staphylococcus aureus and coagulase negative Staphylococcus spp. isolated from patients with acute otitis media." *J. Am. Sci.*, vol. 6, pp. 1022-1033.

[24] Adegoke, A. A. and Okoh, A. I., 2011. "The in vitro effect of vancomycin on multidrug resistant Staphylococcus aureus from hospital currency notes." *Afr. J. Microbiol. Res.*, vol. 5, pp. 1881-1887.

[25] Gad, G. F. M., Abd El-Ghafar, A. E. G. F., A., E.-D. R. A., and Hashem, Z. S., 2010. "Epidemiology and antimicrobial resistance of staphylococci isolated from different infectious diseases." *Brazil. J. Microbiol.*, vol. 41, pp. 333-344.

[26] Onyeadi, D. and Agbagwa, O., 2019. "Plasmid curing in multi-drug resistant hospital and community uropathogenic Escherichia coli." *Journal of Applied Science and Environmental Management*, vol. 23, pp. 29-34.

[27] Finberg, R. W., Moellering, R. C., Tally, F. P., Craig, W. A., Pankey, G. A., and Dellinger, E. P., 2004. "The importance of bactericidal drugs: future directions in infectious disease." *Clinical Infectious Diseases*, vol. 39, pp. 1314–1320.

[28] Li, X. Z. and Nikaido, H., 2009. "Efflux-mediated drug resistance in bacteria: an update." *Drugs*, vol. 69, pp. 1555–1623.