COMPARATIVE STUDY ON THE EFFECTIVENESS OF DIFFERENT BRANDS OF SELECTED ANTIBIOTICS AGAINST ISOLATES OF *Staphylococcus aureus*

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Abstract: This study is to compare the effectiveness of three (3) different commercial brands of antibiotics (the antibiotics selected for the comparison include: Amoxicillin, Ciprofloxacin and Erythromycin) against the standard (NCTC 8854) and two (2) clinical *Staphylococcus aureus* isolated from wounds of two different patients attending Bethel Hospital Wukari, Taraba State. The brands of antibiotics selected and used for this work were marked as Brand 1, 2 and 3. The methods of assay used were, Dilution Test for the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) following these concentrations: 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} mg/ml along with positive and negative control for all the antibiotics. Disc Diffusion Test was carried out to determine the Antibiotic Susceptibility Test (AST). The discs used were locally produced with the concentration of 1 mg/ml for each of the antibiotics. Brand 3 antibiotics were found to be the most effective against all the isolates with MIC as low as 10^{-3} mg/ml and MBC of 10^{-1} mg/ml. There was no much differences in the AST of the different brands. Ciprofloxacin showed up as the antibiotic with better MIC and MBC. Therefore, in comparing the effectiveness of the three (3) different commercial brands of antibiotics; the Brand 3 is the most effective brand while ciprofloxacin is the most effective antibiotic.

Keywords: Amoxicillin, antibiotic, brand, ciprofloxacin, erythromycin, *S. aureus*

Introduction

The emergence of antibiotic resistance in the management of diseases and infections is a serious public health problem and the rapid spread of multidrug-resistant bacterial strains seems to be the most frightening episode now (WHO, 2017). This is particularly seen in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake drugs of questionable quality in circulation (El-Astal, 2005). The levels of potency of these antibiotics vary according to the different manufacturers. Some survey confirmed that some of the brands of antibiotics sold in Nigeria may be fake or adulterated and do not contain the acclaimed quantity of active ingredients (Nkang *et al*, 2010). This could be one of the major factors responsible for the increasing antimicrobial resistance especially in developing countries (Zhanel, 2005). Resistance development is also said to be mostly encountered with the antibiotics frequently used for the treatment of these infections and it varies among communities and also depends on the level of compliance to the right prescription (Tessema*et al.*, 2007; Moges, 2002).

Antibiotics are types of antimicrobial drug used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria (Hugo and Russell, 2004). The introduction of antibiotics to clinical practice represents one of the most outstanding contributions to the treatment of life-threatening infectious diseases. However, due to their extensive usage, numerous resistance mechanisms have emerged and rapidly spreading among bacteria (WHO, 2014). Antibiotics revolutionized medicine in the 20th century and have together with vaccination led to the near eradication of diseases such as tuberculosis in the developed world. However, their effectiveness and easy access led to overuse thereby prompting the development of resistance. It was effective against many bacterial diseases but later becomes ineffective due to bacteria resistance mechanism. Many antibiotics that once cured bacterial diseases no longer work at such; bacteria somehow finding a way to protect itself from antibiotics (WHO, 2014). The antibiotics selected for this work are indicator antibiotics for the treatment of staphylococcal infections. They represent different classes of antibiotics as well have different mechanisms of actions. They include erythromycin which is in the class of macrolides and the mechanism of action is by inhibition of protein synthesis, ciprofloxacin is in the class quinolones, its act by inhibiting the nucleic acid synthesis and amoxicillin is a β-lactam which inhibits cell wall synthesis (Hugo and Russell, 2004).

S. aureus which is a Gram positive, round shaped bacterium is naturally susceptible to virtually every antibiotic that has ever been developed. Resistance is often acquired by horizontal transfer to genes from outside sources, although chromosomal mutation and antibiotic selection are also important. *S. aureus* is remarkable in its ability to acquire resistance to any antibiotic. A fundamental biological property of *S. aureus* is the ability to asymptomatically colonize normal people. Approximately 30% of humans are asymptomatic nasal carriers of *S. aureus* (Gorwitz *et al.*, 2008). *S. aureus* is a normal flora in the body of humans. Carriers of *S. aureus* are at higher risk of infection and they are presumed to be an important source of spread of *S. aureus* strains among individuals. The primary mode of transmission of *S. aureus* is by direct contact, usually skin-to-skin contact with a colonized or infected individual, although contact with contaminated objects and surfaces might also play a role (Miller *et al.*, 2008).

Many substandard antibiotics produced by some pharmaceutical industries are ineffective against many microorganisms, thereby instead of effecting inhibition or killing induces resistance, therefore, this research work is aimed to evaluate the effectiveness of different brands of antibiotics on isolates of *S. aureus.*

Materials and Methods

Antibiotics selection

Three different antibiotics from three different brands of Pharmaceutical Industries in Nigeria were purchased in pharmaceutical shops within Wukari metropolis and used for this work. These antibiotics include: Erythromycin (Brand 1, Brand 2, Brand 3); Ciprofloxacin (Brand 1, Brand 2, Brand 3); Amoxycillin (Brand 1, Brand 2, Brand 3).

Sample collection

Five wound samples were collected from patients attending Bethel Hospital Wukari Taraba State. The surface of the wound was swabbed using a sterile swab stick. The samples were then transported immediately to Microbiology laboratory, Federal University Wukari for the laboratory analysis.

Isolation and identification

The swab containing the sample was dipped into normal saline for few minutes and then streaked on sterile nutrient agar and manitol salt agar plates respectively. The plates were then incubated at 37^0C for 18 to 24 h. The manitol salt agar fermenters which produced golden yellow colonies were further subjected to Gram staining and other standard microbiological identification techniques according to Cheesbrough (2006). A standard culture of *S. aureus* was collected from the Microbiology laboratory Federal University Wukari, purified and confirmed for the research along with the clinical isolates.

Dilution test for determination of minimum inhibitory concentration

Serial dilutions of each of the antibiotics were made with the following concentrations; 1, 0.1, 0.01, 0.001 and 0.0001 mg/ml in five different test tubes for each of the antibiotics against the three isolates. The tubes were inoculated and incubated at 37^0C for 24 h. Two test tubes were also prepared as positive control (Nutrient broth and antibiotic only) and negative control (Nutrient broth and test organism only). Broth tubes that appeared turbid after 24 h as compared to the positive control indicates bacterial growth while tubes that remained clear after 24 h as compared to the positive control indicates no growth. The lowest concentration among the tubes without turbidity was chosen as the minimum inhibitory concentration (Lalitha, 2005; Hugo and Russell, 2004).

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined by inoculating via swabbing method all clear (no growth) tubes from the minimum inhibitory concentration test tube onto a separate sterile Mueller-Hinton agar plate. After incubation at 37^0C for 18 h, the least concentration that produced no growth was considered as the minimum bactericidal concentration (Lalitha, 2005; Hugo and Russell, 2004).

Preparation of antibiotic disc

Whatman filter paper no. 1 was used to prepare the discs. It was cut to approximately 6 mm in diameter using perforator, wrapped with foil paper and sterilized in an autoclave. The sterilize filter paper discs were then placed in each of the diluted concentration (1 mg/ml) of the antibiotics for about 30 min. After which they were removed and placed in a petridish. The discs were kept in an oven for a short time to dry and stored in a cool dry place (Lalitha, 2005).

Antibiotic disc diffusion test (AST)

Using an aseptic technique, a sterile swab was dipped into the inoculum and spread on a sterile Mueller Hinton agar plates to form a bacterial lawn. The prepared discs were then placed on the agar plates. The plates were incubated for 18 to 24 h at 37^0C after which the reading was taken by measuring the zones of inhibition using a transparent ruler (Hugo and Russell, 2004).

Results and Discussion

Out of the five clinical wound samples that were collected, two (2) isolates were confirmed as *S. aureus*. Table 1 showed the minimum inhibitory concentration of the antibiotics against the standard isolate of *S. aureus*, clinical isolate 1 and clinical isolate 2. Antibiotics from Brand 3 showed better activity against the standard isolates and clinical isolate 1 by having the least concentration to inhibit growth compared to the other brands. Brand 3 and 2 showed better activity in amoxicillin against clinical isolate 2.

The MIC results obtained from this study showed clearly the difference in the effectiveness of antibiotics from different brands. The antibiotics from Brand 3 showed a better MIC with the least concentration of 0.001 mg/ml against both the standard and the clinical isolates. Therefore, Brand 3 has the most effective antibiotics for inhibiting the growth of *S. aureus* compared to the remaining two brands. The differences in the effectiveness of the antibiotics from these companies could be a product of the differences in their constituents and active ingredients, the quality of the production materials and as well as the standard of their production processes. The most effective of the three antibiotics irrespective of their brand against the standard isolate of *S. aureus* was ciprofloxacin with the least MIC of 0.001 mg/ml as shown in Table 1 and on the clinical isolates was ciprofloxacin and amoxicillin with least MIC of 0.001 mg/ml. The MIC value of the most effective antibiotics in this work is lesser and better compared to the report of Masroor *et al*. (2009) where the MIC of amoxicillin against *S. aureus* was 0.06 mg/ml. The environmental factor and sources of the isolates might have played a significant role in this variation.

Table 1: MIC of some commercial antibiotics against isolates of *S. aureus*

Antibiotics (mg/ml)	Brand 1			Brand 2			Brand 3		
	S	C1	C2	S	C1	C2	S	C1	C2
Erythromycin	0.1	0.1	0.1	1	0.1	0.1	0.1	0.1	1
Ciprofloxacin	0.1	0.1	0.1	0.1	0.1	0.1	0.001	0.01	0.1
Amoxycillin	0.1	1	0.1	1	0.1	0.01	0.001	0.001	0.001

S = Standard isolate, C1 = Clinical isolate 1, C2 = Clinical isolate 2

Table 2: MBC of some commercial antibiotics against isolates of *S. aureus*

Antibiotics (mg/ml)	Brand 1			Brand 2			Brand 3		
	S	C1	C2	S	C1	C2	S	C1	C2
Erythromycin	1	>1	>1	1	>1	>1	1	0.1	0.1
Ciprofloxacin	>1	0.1	0.1	0.1	>1	0.1	1	0.1	0.1
Amoxycillin	1	>1	>1	>1	>1	>1	1	>1	>1

S = Standard Isolate, C1 = Clinical Isolate 1, C2 = Clinical Isolate 2

Table 2 showed the MBC of the antibiotics against the standard isolate, clinical isolate 1 and clinical isolate 2 of *S. aureus.* All the brands showed a very closely related values for the MBC with the least concentration in ciprofloxacin (0.1 mg/ml) of Brand 2 against the standard isolate. The MBC of the antibiotics against clinical isolate 1 showed Brand 3 to have a better MBC value with the least concentration to kill the bacteria than the other brands and ciprofloxacin had a better activity against the isolate in Brand 1 than the other antibiotics. The Table 2 also showed the MBC of the antibiotics against clinical isolate 2 of *S. aureus.* Brand 3 still showed better activity than the others and ciprofloxacin was found to have a better activity against the isolate in Brand 2 than the other antibiotics.

The MBC and the MIC still followed the same trend of activity and effectiveness, as Brand 3 antibiotics were the most effective and ciprofloxacin with better activity among the antibiotics. The results of the MBC buttress and validate that Brand 3 antibiotics are more effective than the other brands. This could be traced back to the standard and quality assurance maintained by these companies. Nkang *et al.* (2010) stated that, the level of potency of antibiotics varies according to the different manufacturers. Some survey confirmed that some of the brands of antibiotics sold in Nigeria may be fake or adulterated and do not contain the acclaimed quantity of active ingredients (Nkang *et al.*, 2010).

The better activity displayed by ciprofloxacin through the MIC and MBC as seen in this work is in agreement with Sharma *et al.* (2009) who stated that, ciprofloxacin, a member of the quinolones family of antibiotics, has a wide spectrum of activity for both Gram positive and Gram negative organisms. Hugo and Russell (2006) also stated that the newer fluoroquinolone derivatives which include ciprofloxacin show

superior activity against *Enterobacteriacease*, *Ps. aeruginosa* and *Staphylococci*.

Figure 1 showed the locally prepared disc diffusion test against standard isolate of *S. aureus*. Brand 1 had the better zones of inhibition ranging from 20 to 28 mm. Fig. 2 showed the prepared disc diffusion test against clinical isolate 1 of *S. aureus*. All the brands had no much difference in their zones of inhibition. Fig. 3 showed the prepared disc diffusion test against clinical isolate 2 of *S. aureus* and all the brands still had no much difference in their zones of inhibition which range from 18 to 26 mm. The similarity in the zones of inhibition noticed in the AST indicates and shows some level of uniformity among the manufacturers. Though ciprofloxacin created the largest zones of inhibition in all the brands but there is no need of comparing the activities of the three (3) antibiotics because they all have different recommended standard disc strength commensurate to their selective toxicity in human system which was not captured in the preparation of the discs.

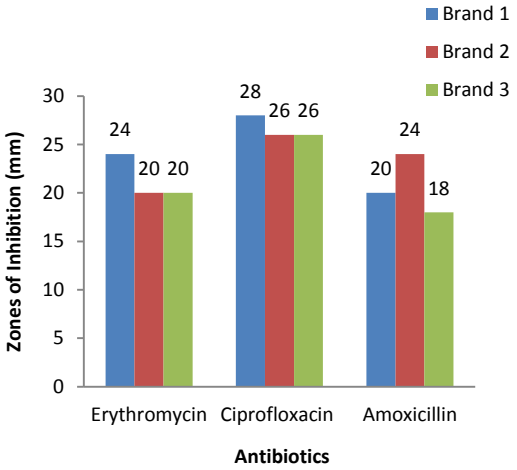

Fig. 1: Locally prepared disc diffusion test against standard isolate of *S. aureus*

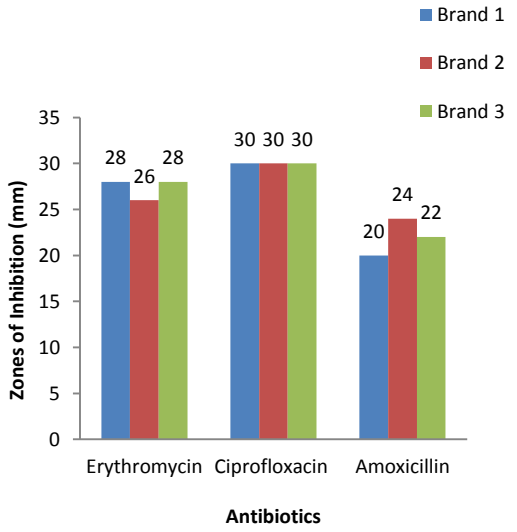

Fig. 2: Locally prepared disc diffusion test against isolate 1 of *S. aureus*

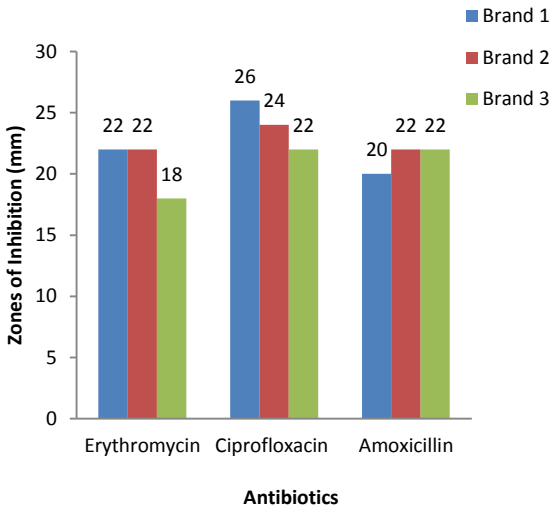

Fig. 3: Locally prepared disc diffusion test against clinical isolate 2 of *S. aureus*

Using the zones of inhibition as a parameter for comparison for the level of the resistance among the isolates, clinical isolates 2 showed more resistance (smaller zones of inhibition) to the antibiotics as shown in Fig. 3. This variation could be as a result of environmental factors and prior exposure of the patient to these antibiotics before the collection of the samples.

Conclusion

This work has revealed that the effectiveness of antibiotics a times depends on the brand concern. In comparing the effectiveness of the different brands of selected antibiotics against isolates of *S. aureus*, antibiotics from Brand 3 were more effective than the antibiotics from Brand 1 and Brand 2 in both the MIC and MBC. Amongst the three (3) selected antibiotics, ciprofloxacin showed up to be the most effective against *S. aureus* compared to amoxicillin and erythromycin. This work has also shown that, it is practicable and feasible to locally produce antibiotic discs with standard efficacy. The differences witnessed in the potency and efficacy of antibiotics from these 3 brands, calls for serious attention from drug regulatory bodies within Nigeria such as National Agency for Food and Drug Administration and Control (NAFDAC) and World Health Organization (WHO) in intervening and reinforcing their work of surveillance and monitoring of the pharmaceutical industries to avoid departure from the standard.

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