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Comparative In-vitro Activity of Cefuroxime and Augmentin on *Staphylococcus aureus* Isolates From Nostrils of Healthy Undergraduate Students of Ambrose Alli University, Ekpoma

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Abstract

The activities of antibiotics are known to differ in pharmacokinetic, pharmacodynamic as well as in the mode and mechanism of action and this create some difficulties in selecting the preferred antibiotic for different bacterial infections. The situation is further made worsen with the development of antibiotic resistance by many bacterial agents and organisms. Based on this background, this study was undertaken to compare the in-vitro activities of Cefuroxime and Augmentin antibiotics indicated by their minimum inhibitory concentration (MIC) on nasal *Staphylococcus aureus* (*S. aureus*) isolates. The study was conducted in the Microbiology Laboratory of the Department of Microbiology, Ambrose Alli University, Ekpoma in Edo State, Nigeria. The *S. aureus* isolates used were isolated from the nostril of students in the Department of Microbiology following due ethical processes. Following standard laboratory procedures, the minimum inhibitory concentration (MIC) of Cefuroxime and Augmentin were determined in 12 confirmed *S. aureus* isolates and the values recorded and compared using the paired sample 't test' of SPSS version 20 at 95% confidence interval. The results showed that Cefuroxime (0.38±0.32 µg/ml) antibiotic has the lowest mean MIC compared to Augmentin (5.16±5.69 µg/ml). While the MIC ranges from 0.02µg/ml to 0.64µg/ml for Cefuroxime antibiotic, it was between 0.05µg/ml to 12.50µg/ml for Augmentin antibiotic. Although both drugs showed antimicrobial potentials against nasal *S. aureus*, based on the findings of this study, Cefuroxime is a better antibiotic for treating respiratory *S. aureus* infection compared to Augmentin.

Keywords

Comparative Antibiotics Activity, Cefuroxime, Augmentin, *Staphylococcus aureus*

1. Introduction

The treatment of infection had a base in folk medicine, the era of chemotherapy was heralded by three land mark discoveries namely; the discovery of sarvasan by Paul Ehrlich in 1908, penicillin by Alexander Fleming in 1927 and sulpha drugs by Gethard Domagk in 1935 (Barriere, 2001). The advent of modern chemotherapy actually began with the pioneer works of Paul Ehrlich at the beginning of 1902, before this time, treatment of infections only has a base in folk medicine some of which are still in practice today (Lindblad, 2008). Although synthetic antibiotic chemotherapy as a source and development of anti-bacterial began in Germany in the late 1880's, Paul Ehrlich in 1902 developed the concept of selective toxicity which today is the cornerstone of chemotherapy. He said that in infected individuals (animals) such chemotherapeutic agents with selective binding properties would therefore be as magic bullets capable of binding the pathogenic cells but, missing the host cells (Gabbum, 1990).

Staphylococcus aureus (*S. aureus*) also known as "golden staph" and "oro staphira" is a facultative anaerobic gram-positive cocci that is frequently found as skin and nasal passages commensal (Kluytmans, 1997). It is an

opportunistic pathogen as it causes infection most commonly in tissues and sites with lowered host resistance as in persons living with diabetes, malnourished aged individuals as well as other chronic disease cases (Brunett, *et al.,* 1996). It causes illness that ranges from minor skin infections to life threatening disease such as Pneumonia, meningitis and is a major cause of nosocomial infection, food poisoning especially food with a high salt or sugar content that is known to favours it growth (Tuo, *et al.,* 1995). Worrisome, is the fact that attempts to control this bacterial by chemotherapy has developed to an increased occurrence of antibiotics resistance (Hiramatsu, *et al.,* 1997).

Of interest are Cefuroxime; a Cephalosporins antibiotic, and Augmentin; a Penicillin and beta-Lactamase Inhibitor Combinations. Cephalosporins are ß-lactam antibiotics *Cephalosporium acremonium;* the first source of the cephaloporins was isolated in 1948. However, Cefuroxime is a second-generation cephalosporin antibiotic (Calderon, 2007). It is given by mouth for the treatment of infections of ears, nose and throat, skin and soft tissue and the upper and lower airways (Kucers, 2004). It is as well available in injections forms that are either intramuscularly or intravenously (Karchmer, 2000) for certain sexually transmitted infections and urinary tract infections when the drug cannot be taken by mouth. Cefuroxime works by interfering with the ability of bacteria to form cell walls

On the other hand, Augmentin is an oral antibiotic combination containing a semi-synthetic antibiotic-amoxicillin (present as amoxicillin trihydrate and amoxicillin sodium) and a β-lactamase inhibitor- clavulanate potassium (the potassium salt of clavulanic acid). By implication, Amoxicillin-clavulanic acid is a single formulation that combines to form a broad spectrum antibiotic activity of a beta-lactam antibiotic by the presence of amoxicillin and a beta-lactamases by the potent inhibitory action of potassium salt of clavulanic acid (Adeleke et al., 2014). Although the combination was invented around 1977/78 by British scientist working at Beecham, it was filed as a drug combination with patent protection in 1979. The synergistic action of the duo favours its popular indication in a wide range of infections that include respiratory infection, genitourinary and abdominal infections, cellulitis, dental infection and animal bites (British National Formulary, 1999). The combination resulted in an increased spectrum of action, restored efficacy against amoxicillin resistant bacteria (British National Formulary, 2001) and increases effectiveness by reducing susceptibility to β-lactamase resistance, for which reason, it is often combined with clavulanic acid (Tortajada, *et al.,* 2008).

Considering the differences in the make-up of Cefuroxime (a single drug and second generation antibiotic) and Augmentin (a combination drug), it is therefore the aim of this study to investigate and compare the efficacy of both drugs indicated by their minimum inhibitory concentrations on nostril *S. aureus* isolates. The study was conducted among apparently health undergraduate students of the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma in Edo State, Nigeria.

2. Materials and Methods

Materials: The materials used in this research work include, measuring cylinder, distilled water, Pasteur pipette, hot air oven, oil immersion, test tube racks, glass slides, masking tape, gloves, markers, detergent, cover slips, microscope, spatula, conical flask, test tubes, autoclave, cotton wool, incubator, aluminum foil, bijoux bottle, inoculating wire loop, Bunsen burner, Petri dishes, disinfectant and weigh balance. The media used were MacConkey agar, Nutrient agar and Peptone water.

Sample source and storage of isolates: Pure culture of twelve (12) gram positive bacteria of *staphylococcus aureus* gotten from the nasal region of healthy susceptible students in Ambrose Alli University, Ekpoma, Edo State, Nigeria, were used for the test. Test isolates were kept on nutrient agar slope and stored at 40^0c before use.

Cleaning and sterilization of equipments used: The glassware used were washed with detergent, water and rinsed in distilled water. All glassware were sterilized using the hot air oven at 160^0c for 1hour, wire loops were sterilized by passing them through a Bunsen burner until red hot before use, other equipment used were also sterilized thoroughly to achieve maximum sterility.

Preparation and sterilization of media: The media were available in the commercially prepared powder forms. Media were reconstituted with water according to manufacturer's guide. Specifically, 28g of nutrient agar powder was weighed and dispensed in 1litre of distilled water and allowed to soak for 10minutes, swirl to mix and them sterilized by autoclaving at 121^0c for 15 minutes. On the other hand, 48.5 grams of MacConkay agar powder was weighed and dispersed in 1 litre of distilled water and was allowed to soak for 10minutes, swirled to mix and then sterilized by autodialing for 15minutes at 121^0c. It was allowed to cool to 47^0c, prior to inoculation, the surface of the agar was dried by parts exposure at 37^0c. Media were dispersed into sterilize Petridis and allowed to solidify at room temperature before use. 15g of peptone water powder were added a litre of distilled water. It was then vigorously mixed and distributed into bijoux bottles, their steadied by autoclaving at 121^0c for 15minute.

Sample Collection: After giving their written/informed consent, nasal swab specimens were collected from the volunteers. The samples collected were then transported to the microbiology laboratory of Ambrose Alli University and analyzed within 24 hours of collection.

Culture and Isolation: The swab samples were streaked unto mannitol salt agar plates and incubated for 18 – 24 hours at 370C. Characteristically golden yellow colour colonies observed after incubation were identified as *S. aureus* and confirmed with coagulase test (Cruickshank *et. al* 1975).

Identification of test isolates: Confirmatory test was done on test isolate, test strain were inoculated into peptone water

and allowed to stay for 2hours on the bench before inoculating onto MacConkey agar. Culture plates were examined and preliminary identification of the isolate was done using their colonial morphology. Biochemical test was then performed.

Morphological characteristics: Isolated colonies from the agar medium were subjected to gram staining using the gram staining technique as described by Christian Gram (1883).

Gram Staining: Differences in gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of gram positive and gram negative organisms during the standing process. Smear of isolates was made on a clean glass slide and fixed by passing the slide through a flame for about 3 times and allowed to cool. The fixed smear is covered with crystal violet stain for 60 seconds. Rapidly wash the stain off with clean water tip off all the water, and then flushed with lugol's iodine for 30-60 seconds, again wash off iodine with clean water. The smear was differentiated briefly with acetone and washed off immediately. It was then counter stained with neutral red and this was allowed to act for 2 minutes and flushed with water. The back of the slide was wiped clean and placed in a draining rack for the smear to air dry, the slides were then examined under the microscope using the oil immersion objective (X100) lens, but was first viewed with the 40X objective to check the staining. The 100X objective showed the morphology and gram reaction of cells gram positive bacterial were recorded as those retaining the colour of the primary stain (crystal violet), while gram negative bacterial were recorded as those retaining the colour of the counter stain (Neutral red).

2.1. Biochemical Tests

Catalase test: This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococci, from non-catalase producing bacteria such as streptococci. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Procedure was as follows; a colony from the pure culture of the organism was emulsified in a drop of normal solution on a slip and a drop or of 3% solution of hydrogen peroxide (H_2O_2) was added to the suspension of the organism. Catalase production from grain positive bacterial was indicated by the production of effervescence or bubbles whereas a negative result showed no gas bubbles or effervescence.

Coagulase test: This test is used to identify *S. aureus* which produces the enzyme coagulase. The procedure was as follows; an inoculum was taken from a pure culture and emulsified in a loopful of normal saline on a clean slide until a homogenous suspension is obtained. A drop of human plasma was added and stirred for 5 seconds. The production of coagulase enzyme was indicated by clotting or coagulation of plasma, which is seen by granule formation while the absence of granules, indicates a negative result.

Sugar fermentation: The sugar used was lactose, glucose, maltose, sucrose and mannitol. In brief, 19% solution of the series of sugars were prepared in peptone water to which neutral red indicator had been added and was sterilized in bijoux bottles containing Durham's tubes. Inoculums was taken from a pure culture and inoculated into peptone water and incubated at 37^0c for 3-4 and after incubation a sterile wire loop was used to inoculate the peptone water culture into sterile sugar solution and the inoculated sugars were incubated at 37^0 for 24 hours. Utilization of sugar was indicated by change in color from red to pink or yellow due to acid production, while gas production was indicated by a space in the Durham's tube. No colour change or gas formation indicates a negative result.

2.2. Determination of Minimum Inhibitory Concentration (MIC)

Tube Dilution Method was use to determine MIC. The stock solutions of each antibiotic (Augmentin and Cefuroxime) were prepared. A row of 10 sterile test tubes were set up on test tube rack. 1ml of sterile peptone water was dispensed into each test tube. Dispense 1ml aliquot from standard solution to the 1st test tube. A drop of in 100 dilution of an overnight broth culture of the test organism was added into each test tube. Test tubes were incubated at 37^0c for 18-24 hours. At the end of the incubation period, the test tubes were observed for turbidity. The last test from the left without visible turbidity was regarded as the one with the concentration of the MIC. This was done for each of the antibiotic. This was repeated three times and the average documented and simple descriptive statistical analysis was performed.

3. Results and Discussion

Students were chosen as the target populations because they are believe to practice good hygiene. The identification of *Staphylococcus aureus* isolates from the nasal region of apparently healthy students in this study justified the fact that *Staphylococcus aureus* is a human commensal and can be asymptomatic. This finding is in accordance with the reported of other studies where *Staphylococcus aureus* infection was reported in population of healthy students (Baliga et al., 2007; Lamikanra et al., 2006). Also, the spreading of *Staphylococcus aureus* infection between health workers and patients and vice versa and among medical students have been documented (Shanmugam et al., 2009; Adesida et al., 2007; Santhosh et al., 2007).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. It is important in diagnostic laboratories to confirm resistance or sensitivity to an antimicrobial agent before the clinician prescribes. In addition, it is important in monitoring the activity of new antimicrobial agents and to determine their relevance in day to day prescription for treatment in clinical use (Andrews, 2001). A lower MIC is an indication of a better antimicrobial agent. Table 1 shows the Minimum Inhibitory Concentration (MIC) of each *S. aureus* to the antibiotics of study. As shown in table 1, all the nasal *S.*

aureus isolates were sensitive to Cefuroxime and Augmentin at varying concentrations. However, the MIC ranges from 0.02µg/ml to 0.64µg/ml for Cefuroxime antibiotic, it was between 0.05µg/ml to 12.50µg/ml for Augmentin antibiotic. Indeed previous studies have shown Cefuroxime (Podolsky and Lawrence 1998) and Augmentin (Tortajada *et al.,* 2008) to have antibiotic potentials to most organisms including *S. aureus*.

Table 1. *Minimum Inhibitory Concentration (MIC) of each S. aureus in the antibiotics of study.*

Isolates	Concentration of Augmentin (µg/ml)	Concentration of Cefuroxime (µg/ml)
1	6.25	0.64
2	12.5	0.64
3	0.78	0.02
4	0.05	0.02
5	12.5	0.02
6	12.5	0.64
7	0.1	0.64
8	0.78	0.02
9	3.125	0.64
10	0.78	0.02
11	12.5	0.64
12	0.05	0.64

Comparatively however, Cefuroxime (0.38±0.32 µg/ml) antibiotic has the lowest mean MIC compared to Augmentin (5.16±5.69 µg/ml). This is shown in table 2 which indicates the mean Minimum Inhibitory Concentration (MIC) of Cefuroxime and Augmentin to *S. aureus* as well as the statistical analysis. Considering the fact that a lower MIC is an indication of a better antimicrobial agent, the observation from this study showed that Cefuroxime is a better antibiotic for nasal *S. aureus* isolates compared to Augmentin. This is further justified by the different in MIC values, differences in minimum and maximum MICs as well as the statistical significant different ($p<0.05$) in mean MIC between the two drugs. It is known that the combination therapy in Augment with the help of the clavulanic acid increases the effectiveness of the amoxicillin by reducing the drug susceptibility to beta-lactamase resistance (Tortajada *et al.,* 2008), this study is not in support based on the observed differences in MIC between the two drugs.

Table 2. *Mean Minimum Inhibitory Concentration (MIC) of Cefuroxime and Augmentin to S. aureus and statistical analysis.*

Variables	Concentration of Cefuroxime (µg/ml)	Concentration of Augmentin (µg/ml)
Min	0.02	0.05
Max	0.64	12.50
Differences in Min and Max MICs	0.62	12.45
Mean±SD	0.38±0.32	5.16±5.69
P value	0.013	
Remark	P<0.05	
Interpretation	There is a statistically significant different	

Values are mean ± SD. CI= 95% and error of 5%.

This study showed that Cefuroxime has higher antibiotic potential compared to Augmentin and this finding is in agreement with previous study by Orhue and Momoh (2012) which showed Susceptibility rate of isolated *Staphylococcus aureus* to be 5.9% for Augmentin and 41.2% for Cefuroxime. The observed antibiotic potential of Cefuroxime is in support of the fact by Agbonlahor and Adegbola (1996), that it is an alternate drug of choice when patients are allergic to the Penicillins or when there is a need to overcome beta-lactamase inactivation. This better antibiotic potency of Cefuroxime than Augmentin has also been shown on *Escherichia Coli* isolates from suspected urinary tract infection (Momoh et al., 2011).

Conclusively, judging by the findings of this study, Cefuroxime is a more powerful antibiotic against nasal *Staphylococcus aureus* compared to Augmentin. Despite the synergistic action of amoxicillin (a semi-synthetic antibiotic) and clavulanate potassium (a β-lactamase inhibitor), thought to give Augmentin a superior antibacterial activity, its inhibitory action was not as potent as Cefuroxime against respiratory *Staphylococcus aureus* infections. Although the synergistic actions of the both combination in Augmentin may favours its indications as a broad spectrum antibiotic considering that β-lactamase-producing bacterial species not susceptible to amoxicillin can be susceptible to the inactivated action of β-lactamase inhibitor-the clavulanate potassium content. The findings of this study therefore intensify the need for bacteriology diagnosis, antibiotic susceptibility testing and monitoring of the activity of antimicrobial agents relevance in day to day prescription. Hence, there seems to be no reason, therefore, for a physician preference of a certain brand to other generic counter parts without proven antibiotic susceptibility testing. On the other hand however, bias for a particular brand or its generic form based on trust and experience due to its long term use with appreciable treatment success is inevitable.

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