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Minimum Inhibitory Concentration of Augmentin on Nasal *Staphylococcus aureus* Isolates Among Undergraduate Students

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Abstract

Although *Staphylococcus aureus (S. aureus)* can be a human commensal, it is a cause of bacterial infections and carries mortality rate of over 30% despite the advancement in the 21st century therapeutics. This is in addition to the growing prevalence of drug resistance that has made treatment with antibiotic to become more problematic. To fight this growing problem of antibiotic, antibiotics combination therapy was adopted as a strategies and this lead to several combination therapy. This study therefore investigates the Minimum inhibitory concentration (MIC) of an antibiotics combination therapy such as Augmentin on nasal *S. aureus* isolates. Of interest however is Augmentin; an oral antibacterial combination consisting of amoxicillin and clavulanate potassium. The study was conducted among apparently healthy undergraduate students of Ambrose Alli University, Ekpoma. Following standard laboratory procedures, biochemical characterization and Augmentin antibiotic susceptibility testing were carried out on 12 samples of *S. aureus* isolates. The results showed that nasal *S. aureus* isolates were positive to coagulase, mannitol, sucrose and catalase but negative to indole, urease and oxidase tests. Overall, all the isolates were susceptible to varying concentrations of Augmentin antibiotic with the minimum MIC and maximum MIC observed at 0.05µg/ml and 12.5µg/ml respectively. The mean MIC of Augmentin antibiotic was 5.16±5.69µg/ml. There was a statistical significant different in the MIC between the 12 samples. The wide variation in the minimum and maximum MICs (12.45µg/ml) with Augmentin antibiotic suggests a wide variation in dosage of Augmentin antibiotic capable of inhibiting the growth of microorganism such as *S.aureus* after overnight incubation.

Keywords

Augmentin, Minimum Inhibitory Concentration, *Staphylococcus aureus*, Undergraduate Students

1. Introduction

Staphylococcus aureus (S.aureus) is a common cause of bacterial infections and carries a higher mortality rate that is currently 20-40% despite appropriate treatment (Brown et al., 2013; Melzer and Welch, 2013). It is found in normal flora of humans and among the leading Gram positive bacteria (GPB) causing diseases in humans and animals, especially in the skin, nasal vestibule as well as the axilla, umbilicus, perineal region and mammary folds (Shanmugam et al., 2009). Although *S. aureus* can be a human commensal, it is a potentially lethal opportunistic pathogen causing varieties of community-acquired and hospital acquired bacterial infections. According to Shanmugam et al. (2009), it is the main cause of opportunistic infections, common diseases such as various types of skin infections including Staphylococcal scalded skin syndrome (SSSS), Osteomyelitis, Meningitis, Pneumonia, Septicemia, and Gastroenteritis. It can be acquired from different sources that include patients, and hospital staff; mainly through their hands and also from their normal flora, and probably the main reasons why the infections occur regularly in hospitalized patients and have severe consequences (Yzerman et al., 1996; Kluytmans et al., 1995).

Of concern, is the fact that carriers can also be healthy human or animal host, carrying potentially pathogenic microorganisms without their knowledge or showing any clinical signs and symptoms of illness (Nester et al., 2004). In fact, report has it that the carrier rate of *S. aureus* in the

nasal canal of healthy individual range from 20-30% with more likelihood of spreading from healthy carriers among the hospital health care personnel from their hands, nose or throat by way of touching, sneezing, talking and coughing (Shanmugam et al., 2009). Indeed several studies have clearly indicate the serious problems posed by *S. aureus* infections spreading between health workers and patients and vice versa; specifically among medical students (Shanmugam et al., 2009; Adesida et al., 2007; Santhosh et al., 2007) as well as population of healthy students (Baliga et al., 2007; Lamikanra et al., 2006).

Worrisome, the emergent antibiotic resistance by several agents has remains a major problem in medical practice (Hannah et al., 2008; Arshad et al., 2012). To address this growing problem of antibiotic resistance, antibiotics combination therapy was adopted as a strategies and this led to several combination therapy. Of interest however is Augmentin; an oral antibacterial combination consisting of the semi-synthetic antibiotic amoxicillin (present as amoxicillin trihydrate and amoxicillin sodium) and the β-lactamase inhibitor clavulanate potassium (the potassium salt of clavulanic acid). Amoxicillin-clavulanic acid combines the broad spectrum antibacterial activity of a beta-lactam antibiotic, amoxicillin and the potent inhibitory action of potassium salt of clavulanic acid on beta-lactamases in a single formulation (Adeleke et al., 2014). The synergistic action of the duo favours its popular indication in a wide range of infections that include respiratory, genitourinary and abdominal infections as well as cellulitis, dental infection and animal bites (British National Formulary, 1999). Based on this broad spectrum property of antibiotics combination therapy, this study therefore investigates the Minimum inhibitory concentration (MIC) of an antibiotics combination therapy such as Augmentin on nasal *S. aureus* isolates among apparently healthy undergraduate students of Ambrose Alli University, Ekpoma.

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. Specificallt, 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 were lactose, glucose, maltose, sucrose and mannitol, 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. The stock solution of the antibiotic (Augmentin) was 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 and this was recorded and repeated three times and the average documented and simple descriptive statistical analysis was performed.

Table 1. *Biochemical characteristic of the nasal isolates of S. aureus.*

Bacterial spp.	Biochemical test									
	Coagulase	Mannitol*	Sucrose*	Lactose*	Glucose*	Maltose*	Indole	Urease	Oxidase	Catalase
S. aureus	+	+	+	NA	NA	NA	-	-	-	+

Key: * = Fermentation test; + = Positive; - = Negative, NA = Not available. Note: cultural characteristic of *S. aureus* is Golden Yellow characteristic

3. Results

Twelve isolates of *Staphylococcus aureus* from the nasal region of healthy susceptible students of Ambrose Alli University was used for this study. Table 1 shows the biochemical characteristics of the *S. aureus* isolated from the students. It was shown that the nasal *S. aureus* isolates yield growth on Coagulase, mannitol, sucrose and catalase tests and as such were positive to these tests but did not yield growth in indole, urease and oxidase tests as thus were negative. The biochemical analysis showed that the nasal *S. aureus* isolate was β-Lactamase producer and cultural characteristic of *S. aureus* is Golden Yellow characteristic.

Table 2 shows minimum inhibitory concentration (MIC) of the different nasal *S. aureus* isolate to Augmentin. Analysis showed that the minimum and maximum MICs were 0.05µg/ml and 12.5µg/ml respectively but the mean MIC was 5.16±5.69µg/ml. Analysis of the results showed that the different in minimum and maximum MIC was 12.45µg/ml. Results also showed that there was a statistically significant different ($p<0.05$) in the MICs between the minimum and maximum MICs of the 12 samples of nasal *S. aureus* isolate.

Table 2. *Minimum Inhibitory Concentration (MIC) of S. aureus to Augmentin antibiotics.*

Variables	Concentration of Augmentin (µg/ml)
Min MIC	0.05
Max MIC	12.5
Difference in MICs	12.45
Mean±SD MIC	5.16±5.69
t value	3.141
Sig. (2-tailed)	0.009
Remark	P<0.05
Interpretation	There is a statistically significant different

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

4. Discussion

The biochemical characteristics of nasal *S. aureus* isolated reported in the present study agrees with those identified in a study conducted among patient attending the Irrua Specialist Teaching Hospital, Edo state, Nigeria (Orhue and Momoh, 2012). The study showed that *S. aureus* was positive for β-Lactamase indicated via the biochemical methods but negative to iodometric biochemical methods. This indicated that the enzymes produced by the *S. aureus* isolates were β-Lactamase and not acylase. This conclusion is based on the fact according to Plested et al. (1983) that β-Lactamase gives positive result to microbiological and acidimetric methods and negative result to iodometric method. In addition, a study conducted in Nigeria has previously reported that 100.0% of hospital isolated *S. aureus* was β-Lactamase producer (Kolawole et al., 1992), by implication, the findings of this present study is in accordance.

Also a finding of this study is the wide variation (12.45µg/ml) between the minimum and maximum MICs of Augmentin antibiotic. This wide variation suggest wide effective dose of the drug. In line with the present finding, a study has shown a wide range from 2.5µg/ml to 62.5µg/ml for different *S. aureus* isolated (Adeleke et al., 2014). In fact, the study reported MICs for different brands of Augmentin and these ranges from 1.25µg/ml to 62.5µg/ml for five different *S. aureus* isolated. In another line of thought, the wide different in MIC of Augmentin antibiotic to nasal *S. aureus* isolate indicate drug resistance. In agreement with this assertion, a recent study has reported a 93 to 90% resistance of *S. aureus* nasal isolate to Augmentin (Emeka et al., 2013). This might be due to antibiotic misuse or abuse, as suggested by a study which reports that Penicillins are the most misused antibiotics amongst Nigerian communities (Olayemi et al., 2010). Furthermore Adegoke and Komolafe (2009) reported a 72% penicillin resistance on *S. aureus* isolates, thus presenting a worrisome situation as penicillin is still being used in the treatment of *Staphylococcal* infections. However others were of the view that this resistant *S. aureus* strains have emerged from the hospitals and spread to the community (Couto et al., 2000).

Considering the fact that Augmentin is an antibacterial combination of the semi-synthetic antibiotic amoxicillin and the β-lactamase inhibitor clavulanate potassium, it is expected that it should bear a more specific MIC with very close difference. Clavulanic acid is class A serine β-Lactamase inhibitor, the suicide inactivator has mechanism of inhibiting β-Lactamase similar to that of semi-synthetic inhibitors. The combination inhibits growth of bacteria producing β-Lactamase such as the common TEM, SHV and OXA enzymes. All these are used successfully in combination with penicillin such as in Clavulanic acid–amoxycillin, sulbactam–ampicillin, tazobactam–piperacillin. These are often in plasmid coded β-Lactamase in gram-negative bacteria (Bush, 2003). When such combination was first introduced, multiple β-Lactamase production was rare even in organisms with plasmid encoded β-Lactamase genes. Typically, extended spectrum β-Lactamase (ESBL) is mutant plasmid mediated β-Lactamase, derived from older broad spectrum β-Lactamase which has extended substrate profile (Thomson, 2001).

This was however not the case based on the finding of this study. In another line of thought, the differences in MICs between the present study and previous study may be related to the issue of quality of the drugs. This assertion is based on the fact that there are proliferating reports that 68% of drugs sold in the Nigeria market are counterfeit (Odulaja *et al.,* 2012). Although poor-quality of drug has been a global public health concern, and this is more worrisome for such sensitive drugs as antibiotics used in managing infections. The growing prevalence of antibiotic resistance may also be owned to the issue of counterfeit drugs and poor or substandard drugs. Indeed it has previously been reported that substandard antibiotics account for the continued emergence of bacterial resistance. In another line of thought, studies have shown that misuse of antibiotics is a main cause of antimicrobial resistance (Okeke et al., 2007; Ojo et al., 2008) and Nigeria has a high rate of antibiotic misuse as well as high prevalence of self-medication use (Sapkota et al., 2010). This again may be the cause of the growing prevalence of antibiotic resistance. Thus intensifying the importance of diagnostic laboratories to confirm resistance or sensitivity of antimicrobial agents before they are clinician prescribes.

The reason for different brands of drugs in the market is to increase affordability for consumers and promote competition. By implication, to ease patients' healthcare cost burden since brands contain similar generic drugs and are bioequivalent (contain the same active ingredients) and as such similar pharmacological effect. However, upon testing the bioequivalence of some brands of amoxicillin-clavulanate based on physicochemical characteristics, Odulaja *et al* (2012) reported that 16.7% failed standard of acceptability. This may again strengthen the fact that the growing antibiotic drugs resistance is due to counterfeit and substandard types. This may not be unrelated to the reported by various workers that bacterial specifically *S. aureus* spp. have gain remarkable ability to acquire antibiotic resistance contributing to its emergence as an important pathogen in a variety of setting (David et al., 2006; Sampathukumar, 2007; Hotu et al., 2007).

The need for conducting in-vitro antibiotic susceptibility tests prior to drug administration is an important parameter in determining performance following administration and this is justified in the spate of antibiotic resistance leading to therapeutic failure. A study has previously reported an in-vitro susceptibility test to 28 different isolates to be completely resistant to augmentin (Adeleke et al., 2014). Many antibiotics such as augmentin, erythromycin, chloramphenicol, ampicillin and tetracycline are normally prescribed for the treatment of otitis media. However bacterial resistance to these antibiotics has become an increasing problem in the treatment of otitis media (Alsaimary et al*.,* 2010). The results from this study clearly support this assertion. Augmentin has also been reported to

be resistance to other bacterial causative agents such as *Proteus* spp (Bahashwan and El Shafey, 2013) and thus the need for attention to be paid to the increasing resistance of many antibiotics especially Augmentin. Not only are *S. aureus* potential causes of infections but also potential reservoirs of resistance genes that could be transferred to other bacterial pathogens. The wide variations in MICs in this study and the reported high levels of β-lactamase production and multi-drug resistance of bacterial isolates are indications of increase in the resistance menace reported by many studies (Feglo et al., 2010). However, Augmentin has been shown to be efficacious in the management of furunculosis infections (a skin infection caused by *Staphylococcus aureus*) (Okunye et al., 2011).

5. Conclusion

Conclusively, although this study showed Augmentin to have antibacterial activities on nasal isolate of *S. aureus*, there is a significant variation in the lowest concentrations of Augmentin capable of inhibiting the visible growth of nasal isolate of *S. aureus* after overnight incubation. Thus, *S. aureus* isolated from nasal region (of respiratory origin) may present wide variation of susceptibility to Augmentin and by implication increased Augmentin resistivity to the bacterial agent. However, increased dosage and long duration of used may be more susceptible and give a promising bacteriacidal action to nasal isolate of *S. aureus* by Augmentin antibiotics.

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