# Susceptibility of some Gram-positive and Gram-negative bacteria against some brands of amoxicillin-clavulanic acid

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**Abstract:** Antimicrobial resistance continues to increase in leaps and bounds cutting across different classes of antibiotics. Among the strategies adopted to combat the menace is combination drug therapy. Amoxicillin-clavulanic acid combination finds application in this regard. Branded antibiotic combinations are equally on the increase thereby posing a problem of making the right choice among the different brands by the medical personnel. This study evaluated the antimicrobial activity of five different brands of amoxicillin-clavulanic acid against twenty-eight Gram-positive and Gram-negative bacterial species from four Genera. All the isolates produced beta-lactamase and were mostly multidrug-resistant. The Gram-negative bacterial isolates showed least resistance rates to ofloxacin. Seven of the 10 highly resistant strains revealed one copy each of plasmid DNA. Generally, curing of plasmid DNA was obtained at 200 µg/ml of ethidium bromide in all the six isolates tested using 30 µg/ml and 12 µg/ml of amoxicillin-clavulanic acid. Minimum Inhibitory and Minimum Bactericidal Concentrations obtained by tube-broth dilution for the Gram-negatives were high compared to the Gram-positives. The study reflects no differences in the potency of the brands of AMC tested and increase in the resistance of all the bacterial isolates to beta-lactam antibiotics.

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**Key word**: amoxicillin-clavulanate, brands, bacteria, susceptibility

## Introduction

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. The synergistic action of the duo favours its popular indication in a wide range of infections due to beta-lactamase-producing bacterial species usually non-susceptible to amoxicillin alone; these include respiratory infection, genitourinary and abdominal infections, cellulitis, dental infection and animal bites (British National Formulary, 1999). Very many members of the Enterobacteriaceae and staphylococci produce beta-lactamase which hydrolyzes beta-lactam ring of the penicillins. Clavulanic acid inhibits majority of the clinically significant beta-lactamases, production of which is either by plasmid or chromosomally-mediated or by both.

In the Nigerian drug market, both the standard innovator drug, augmentin® and the generic forms are available. This allows for affordability to high population of consumers due to competition and thus may ease patients' healthcare cost burden. Brand-name drugs show similar pharmacological effect as the generic drugs and are bioequivalent, since they contain the same active ingredients. Differences may exist only in the manufacturing processes, the physicochemical properties of the drug and the excipient type added which are vital determinants of the efficacy of a brand product (Melissa, 2009; Manish, 2010). There seems to be no reason, therefore, for any physician to prefer prescription of a certain brand to other generic counter parts. 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.

The franchise to manufacture and sell any drug brand is, however, within the confine of various drug regulatory agencies in each country to assure drug quality, safety and potency. Drug regulatory function ensures that counterfeit drugs do not find their way into the market. Nevertheless, there abound reports that 68% of drugs sold in the Nigeria market are counterfeit (Odulaja *et al.,* 2012). Poor-quality medicine is a global public health concern and this is more worrisome for such sensitive drugs as antibiotics used in managing infections. Substandard antibiotics account for the continued emergence of bacterial resistance.

The effort of the nation's drug regulatory body, National Agency for Food and Drug Administration Commission (NAFDAC) in ridding the country of counterfeit or substandard medicines is being complemented with the efforts of researchers on the frequently observed therapeutic failures of drugs by carrying out independent studies to assess the quality of the medicine. For instance, some brands of

ciprofloxacin in the Nigerian markets have been found to be of lesser quality than that required for use in treatment (Adegbolagun *et al.,* 2007; Odeniyi *et al*., 2000). Upon testing the bioequivalence of some brands of amoxicillin-clavulanate based on physico-chemical characteristics, Odulaja *et al* (2012) reported that 16.7% failed.

Microbiological methods are capable of detecting subtle changes in the drugs often missed by the chemical method. Adeleke *et al.* (2010) revealed the disparity in the effectiveness of two brands of cefuroxime and ceftriaxone compared to other five that were tested against Gram-negative isolates. Therefore, this study set out to compare the efficacy of various brands of amoxicillin-clavulanic acid against some bacteria as well as determine the role of plasmid in their antibiotic resistance pattern.

## 2. Materials and Methods

**Bacterial strains**

Twenty eight clinical isolates of Gram-positive and Gram-negative bacteria were obtained from the routine section of the Department f Medical Microbiology and Parasitology, University College Hospital UCH, Ibadan and the laboratory section of St Mary's Catholic Hospital, Eleta Ibadan .The bacteria comprised *Staphylococcus aureus*(5), *Staphylococcus epidermidis* (5), *Escherichia coli* (5), *Proteus mirabilis* (5) and *Pseudomonas aeruginosa* (5) and typed strains *S. aureus* ATCC 25923, *E coli* ATCC 36318 and *P. aeruginosa* ATCC27853.Their identification was done using conventional microbiology methods (Cheesebrough, 2000).

**Antibiotics**

The five brands of amoxicillin-clavulanic acid injection powder used were coded as AUG, OXI, FLM, AMK and AMV. They were all purchased from pharmacy stores in Ibadan, Nigeria.

**Antimicrobial susceptibility testing**

Antibiotic susceptibility pattern of the bacterial isolates was determined using agar disc diffusion method (Cheesebrough, 2000). Against the Gram-negative bacteria, the antibiotics used were: amoxicillin (25µg), co-trimoxazole (25µg), nalidixic acid (30 µg), gentamicin (10µg), ofloxacin (30µg), augmentin (30µg), tetracycline (30 µg) (Abtek Biological Limited) and for the Gram-positives, the antibiotics comprised augmentin, erythromycin (5µg), tetracycline, cloxacillin (5µg), gentamicin, cotrimoxazole and chloramphenicol (30µg). Overnight broth culture of each isolate adjusted to match 0.5 McFarland Standard ($10^8$ CFU/ml) was used to seed well dried Mueller-Hinton agar plates and each plate was incubated at 37$^o$C for 24 h. The plates were observed for zones of growth inhibition. Isolates were classified as resistance (R), intermediate (I) or sensitive (S) according to Clinical and Laboratory Standards Institute guidelines (CLSI) 2009.

**Detection of Beta-lactamase production**

Cell-suspension iodometric method as described by Sykes (1978) was used to detect beta-lactamase production in each of the bacterial isolates. Briefly, cell suspension of density $10^9$cells/ml was homogenized in 0.5 ml phosphate buffered solution containing penicillin G (10000 units or 0.06mg/ml) and left to stand for 1h before two drops of freshly prepared 1% aqueous soluble starch were added. The mixture was shaken gently after which a drop of iodine was added. A change of colour of mixture from blue/blue–black to colourless within 10 mins was positive for beta-lactamase production.

**Determination of minimum inhibitory concentration (MIC)**

MIC of each brand of amoxicillin-clavulanic was determined for the 28 isolates by the tube broth-dilution method (Adeleke *et al.*, 2010). A two- fold serial dilution in freshly prepared nutrient broth of each antibiotic was prepared in graduated decreasing concentrations. To each dilution was added 0.1ml of $10^{-2}$ diluted overnight culture of the isolates. All the tubes were incubated at 37$^o$C for 24 h. Negative control tubes were also prepared by transferring a dilution of each antibiotic from the last dilution into the sterile clean tubes. Control strain was included in the test-run. MIC was taken as the least concentration that inhibited discernible bacterial growth in the dilutions.

**Determination of minimum bactericidal concentration**

All the test mixtures in the tubes that showed no visible growth in the MIC test were sub-cultured on drug-free nutrient agar plates and incubated at 37$^o$C for 24 h. The lowest concentration that inhibited 99.9% of growth on the plate recorded the MBC (Cheesebrough, 2000).

**Curing experiment**

Curing of resistance plasmid in the bacterial isolates was done as described by Yah *et al* 2008 and Akortha *et al* 2010 with slight modification. The cells were grown in nutrient broth containing ethidium bromide at different concentrations of 200µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml for 24h at 37ºC**.** A loopful of each well agitated mixture was sub-cultured on to drug-free nutrient agar plates, followed by antibiotic susceptibility test carried out on distinct colonies that grew from each plate after 24h incubation against amoxicillin-clavulanic acid. The concentrations of the antibiotic used were 1 µg/ml, 5 µg/ml, 10 µg/ml, 12 µg/ml and 30 µg/ml. Presence of zones of growth inhibition around the well indicated plasmid has been cured.

**Plasmid processing**

The method of preparation of plasmid DNA by small-scale boiling lysis was as described by Sambrook and Russel (2006). A single colony of transformed bacteria was inoculated into 2ml of terrific broth containing the antibiotic and incubated at 37ºC for 24h with vigorous shaking**.** 1.5ml of the culture was removed and centrifuged at maximum speed for 30s at 4 ºC. The dry bacterial pellet was collected and then resuspended in 350µl of Sucrose Triton EDTA Tris (STET) HCL to which 25µl of freshly prepared lysozyme was added. The mixture was gently vortexed and placed in boiling water for 40s. The lysate was then centrifuged at maximum speed for 15mins at room temperature. To the supernatant collected into the microfuge tube, 40 µl of 2.5M sodium acetate (pH 5.2) and 420 µl of isopropanol were added in order to precipitate nucleic acid.

The precipitated nucleic acids were recovered by centrifugation at maximum speed for 10 mins at 4ºC after which the supernatant was removed by gentle aspiration. The tube was then allowed to stand inverted on a paper towel to drain all the fluid away. Excess drops of fluid on the wall of the tube were removed using disposable pipette. The nucleic acid was rinsed in 1ml of 70% ethanol at 4ºC and the supernatant was gently removed by aspiration. The open tube was stored at room temperature until the ethanol evaporated and no fluid was visible in the tube. The nucleic acids were dissolved in 50µl of Triton EDTA (pH 8.0) DNA-free RNase A (pancreatic RNase) and the solution was vortexed for a brief period.

The molecular weight of the extracted plasmid was determined by agarose gel electrophoresis run at 75V for 90 mins on 0.8%w/v agarose gel 100ml in single strength Tris borate/EDTA electrophoresis buffer. After electrophoresis, the DNA bands were stained in 0.5 µg/ml ethidium bromide prepared in de-ionized water for 10-15mins at 250ºC

The stained gel was visualized with a short wave UV light trans-illuminator and then photographed. The DNA bands were matched with those of lambda Hind III Digest MW marker in the range 0.1-23.1kb to obtain molecular weight of the DNA bands.

**3. Results**

The identities of the twenty–eight clinical isolates used in the study were confirmed by the conventional methods. All the isolates produced beta-lactamase. The two types of multo-disks used for susceptibility testing (Gram-positive and Gram-negative multo-disks) had in common the following antibiotics: augmentin, amoxicillin, tetracycline, cotrimoxazole and gentamicin. The in- vitro susceptibility test showed that all the 28 isolates were completely resistant to augmentin and amoxicillin. All the 11 Gram-positive bacterial isolates were resistant to cloxacillin. Overall resistance rates of 71.45% to tetracycline and 85.71% to both cotrimoxazole and gentamicin were obtained. The Gram-negative bacteria tested were highly sensitive to ofloxacin, giving a low resistance rate of 23.53% (Table 1).

Of the 28 isolates, twenty were multidrug-resistant. The commonest resistant pattern was amc,am,cxc,ery,tet,gen,cot,chl for staphylococci, while amc,am,nal,tet,gen,cot,nit was the commonest for Gram-negatives mostly found in *Pseudomonas spp* (Table 2). EU2, PR1, PR4 and PR5 showed intermediate sensitivity to nitrofurantoin; SB3, EU3 and PR1 to gentamicin; PS1 and PS4 to nalidixic acid. SU7 and SB3 were intermediately susceptible to tetracycline and erythromycin respectively.

Table 1. Overall antimicrobial susceptibility result for the isolate

| Antibiotics | No of isolates | Resistance | Sensitive | Intermediate |
|---|---|---|---|---|
| Augmentin | 28 | 28(100.00%) | 0(0.00%) | 0(0.00%) |
| Amoxicillin | 28 | 28(100.00) | 0(0.00) | 0(0.00) |
| Tetracycline | 28 | 24(85.71) | 3(10.71) | 1(9.09) |
| Gentamicin | 28 | 20(71.45) | 5(17.86) | 3(10.71) |
| Cotrimoxazole | 28 | 24(85.71 | 4(14.29) | 0(0.00) |
| Erythromycin[p] | 11 | 7(63.64) | 3(27.27) | 1(9.09) |
| Chloramphenicol[p] | 11 | 5(45.45) | 6(54.54) | 0(0.00) |
| Cloxacillin[p] | 11 | 11(100.00) | 0(0.00) | 0(0.00) |
| Nitrofurantoin[n] | 17 | 10(58.82) | 3(17.65) | 4(23.53) |
| Nalidixic acid[n] | 17 | 9(52.94) | 6(35.29) | 2(12.00) |
| Ofloxacin[n] | 17 | 4(23.53) | 13(76.47) | 0(0.00) |

Overall antimicrobial susceptibility result for the isolate

[p] Tested for Gram-positive *S. aureus* and *S. epidermidis*;[n] tested for Gram-negative *P. aeruginosa, E.coli, Pr. mirabilis*

Table 2. Resistance patterns of 28 Gram-positive and Gram-negative bacterial isolates

| Resistant pattern | No of antibiotics class | No of antibiotics | Isolates |
|---|---|---|---|
| amc, am, cxc | 1 | 3 | SU11 |
| amc,am,cxc,ery | 2 | 4 | SB3 |
| amc,am,cxc,cot | 2 | 4 | SU4, SU7 |
| amc,am,cxc,ery,tet | 3 | 4 | SA ATCC 25923 |
| amc,am,cxc,ery,tet,gen,cot,chl | 6 | 8 | SU12, SU9, SU6, SU8, SW1 |
| amc,am,tet | 2 | 3 | EU3 |
| amc,am,tet,cot | 3 | 4 | EU2, PR1 |
| amc,am,tet,gen,cot | 4 | 5 | PS ATCC 27853 |
| amc,am,tet,gen,cot,nit | 5 | 6 | PS1, PS4 |
| amc,am,tet,gen,cot,nit | 5 | 6 | EU4 |
| amc,am,nal,tet,gen,cot | 5 | 6 | PR5 |
| amc,am,nal,tet,gen,cot,ofl | 5 | 7 | EU5 |
| amc,am,nal,tet,gen,cot,nit | 6 | 7 | PR2, PS2, PS3, PS5 |
| amc,am,tet,gen,cot,nit,ofl | 6 | 7 | PR3 |
| amc,am,nal,tet,gen,cot,ofl | 5 | 7 | PR4 |

Key*: S. aureus* (SB3, SW1, S11, SU8), *S. epidermidis* (SU4, SU5, SU12, SU9, SU7) *P. aeruginosa* (PS1-5), *Pr. mirabilis* (PR1-5), *E coli* (EU1-5).
Am =Amoxicillin, amc = augmentin, cot=co-trimoxazole, nal = nalidixic acid, gen = gentamicin, ofl =ofloxacin, tet= tetracycline, , ery =erythromycin, cxc = cloxacillin, and chl =chloramphenicol,

Table 3. Minimum inhibitory concentrations (µg/ml) of the brands of Amoxicillin-Clavulanic Acid against the isolates

| Isolates | Brands of Amoxicillin-Clavulanic Acid | | | | |
|---|---|---|---|---|---|
| | AUG | OXI | FLM | AMK | AMV |
| SU11 | 2.5**(2)** | 1.25**(2)** | 1.25**(2)** | 1.25**(2)** | 1.25**(2)** |
| SB3 | 6.25**(2)** | 6.25**(2)** | 6.25**(2)** | 12.5**(2)** | 12.5**(2)** |
| SW1 | 50**(2)** | 50**(2)** | 50**(2)** | 50**(2)** | 50**(2)** |
| SU6 | 50**(2)** | 50**(2)** | 50**(2)** | 50**(2)** | 50**(2)** |
| SU8 | 62.5**(2)** | 62.5**(2)** | 62.5**(2)** | 62.5**(1)** | 62.5**(2)** |
| S.A ATCC 29523 | 10**(1.25)** | 10**(1.25)** | 10**(1.25)** | 10**(1.25)** | 10**(1.25)** |
| SU4 | 25**(1)** | 25**(1)** | 25**(1)** | 12.5**(2)** | 12.5**(1)** |
| SU5 | 25**(2)** | 12.5**(2)** | 25**(2)** | 25**(2)** | 25**(2)** |
| SU12 | 125**(1)** | 62.5**(1)** | 62.5**(1)** | 62.5**(1)** | 125**(1)** |
| SU9 | 125**(2)** | 125**(1)** | 125**(1)** | 125**(1)** | 125**(2)** |
| SU7 | 250**(1)** | 250**(1)** | 500**(1)** | 250**(1)** | 500**(1)** |
| *E.coli* ATCC36318 | 25**(1)** | 25**(1)** | 50**(1)** | 50**(1)** | 50**(1)** |
| EU1 | 125**(2)** | 125**(2)** | 125**(1)** | 62.5**(2)** | 250**(1)** |
| EU2 | 250**(1)** | 250**(2)** | 250**(2)** | 125**(2)** | 500**(1)** |
| EU3 | >500 | 250 | >500 | 250**(2)** | >500 |
| EU4 | 500**(1)** | 250 | 500**(1)** | 250**(1)** | 500**(1)** |
| EU5 | 250**(1)** | 250**(1)** | 500**(1)** | 250**(1)** | 500**(1)** |
| PS1-**5** | >500 | >500 | >500 | >500 | >500 |
| PR1 | 250**(2)** | 500 | 500 | 250**(1)** | 500 |
| PR2 | 125**(1)** | 500 | 500**(1)** | 125**(1)** | 250**(2)** |
| PR3 | 125**(2)** | 125**(2)** | 500**(1)** | 250**(2)** | 125**(1)** |
| PR4 | 500 | 500 | 500 | 500**(1)** | 500 |
| PR5 | 125**(2)** | 250**(1)** | 250**(2)** | 250**(1)** | 500**(1)** |

Bold type: Values in the bracket are ratio MBC/MIC. Where MBC is > 500 µg/ml, no value is given.

Table 4. Antibiotic sensitivity of the strains of the bacteria isolates after exposure to ethidium bromide

| Conc. EB(µg/ml) | 200 | | | | | 100 | | | | | 50 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Varying Concentrations of amoxicillin-clavulanate (µg/ml) | | | | | | | | | | | | | | | |
| | 30 | 12 | 10 | 5 | 1 | 30 | 12 | 10 | 5 | 1 | 30 | 12 | 10 | 5 | 1 |
| **Bacterial isolate** | | | | | | | | | | | | | | | |
| *S. aureus* **SW1** | | | | | | | | | | | | | | | |
| S1 | 26 | 25 | 18 | 12 | 10 | 24 | 20 | 18 | 15 | 12 | R | R | R | R | R |
| S2 | 30 | 25 | 24 | 22 | 12 | 24 | 22 | R | R | R | R | R | R | R | R |
| S3 | 28 | 25 | 18 | 12 | 10 | 23 | 19 | 18 | R | R | R | R | R | R | R |
| *S. epidermidis* **SU7** | | | | | | | | | | | | | | | |
| S1 | 22 | 20 | 18 | 15 | 13 | 14 | 12 | 10 | R | R | 12 | R | R | R | R |
| S2 | 19 | 17 | 16 | 16 | 15 | R | R | R | R | R | R | R | R | R | R |
| S3 | 34 | 31 | 25 | 21 | 10 | R | R | R | R | R | R | R | R | R | R |
| *E. coli* **EU3** | | | | | | | | | | | | | | | |
| S1 | 23 | 22 | 20 | R | R | R | R | R | R | R | R | R | R | R | R |
| S2 | 24 | 22 | 20 | 18 | R | R | R | R | R | R | R | R | R | R | R |
| S3 | 25 | 22 | 20 | 18 | R | R | R | R | R | R | R | R | R | R | R |
| *P. mirabilis* **PR1** | | | | | | | | | | | | | | | |
| S1 | 20 | 16 | 16 | 15 | R | R | R | R | R | R | R | R | R | R | R |
| S2 | 12 | 10 | R | R | R | R | R | R | R | R | R | R | R | R | R |
| S3 | 12 | 11 | R | R | R | R | R | R | R | R | R | R | R | R | R |
| *P. aeruginosa* **PS1** | | | | | | | | | | | | | | | |
| S1 | 19 | 12 | R | R | R | R | R | R | R | R | R | R | R | R | R |
| S2 | 20 | 13 | R | R | R | R | R | R | R | R | R | R | R | R | R |
| S3 | 18 | 12 | R | R | R | R | R | R | R | R | R | R | R | R | R |
| *P. aeruginosa* ***ATCC27853*** | | | | | | | | | | | | | | | |
| S1 | 16 | 15 | 15 | 15 | 15 | 17 | 16 | 14 | R | R | R | R | R | R | R |
| S2 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| S3 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |

Key: R= resistant

The zone of growth inhibition obtained by the disk diffusion method for augmentin was consistent with the MICs for all the five brands of amoxicillin-clavulanic acid (AMC).The only exception was with SU11 that was fully sensitive to all the brands. The MIC range for brand AUG was 2.5->500 µg/ml and other brands OXI, FLM, AMK, and AMV had similar range of 1.25->500µg/ml. The Gram-positives' MIC values were lower compared to those for Gram-negative bacteria. The pseudomonad strains recorded higher values of MIC and MBC, >500. Only PS2 had MIC value of 500µg/ml. The value of MIC/MBC for the isolate ranged from 1-2.

Six bacterial isolates tested for resistance curing experiment showed elimination of plasmid DNA generally at 200 µg/ml of ethidium bromide (Table 4). One copy of plasmid DNA having molecular weight of 23.130 kb was extracted from each of the six isolates tested.

## 4. Discussion

The need for conducting in-vitro antibiotic susceptibility tests prior to drug administration as an important parameter in determining performance following administration is justified in the spate of antibiotic resistance leading to therapeutic failure. Antibiotic resistance remains a major and emerging problem in medical practice (Hannah *et al.,* 2008; Arshad *et al.,* 2012). The results from this study clearly support this assertion. The beta-lactams, augmentin and amoxicillin were inactive against the bacterial species from four Genera. In the same vein, all the Gram-positive bacteria tested were resistant to cloxacillin. This is consistent with the fact that all the isolates were beta-lactamase producers. Resistance to beta-lactam antibiotics is most often caused by the production of beta-lactamase (Guler *et al*., 2005).

The use of penicillins in the treatment of infections caused by the bacterial species tested in our locality should be a follow-up to laboratory testing. Aibinu *et al.* (2003) noted that beta-lactams are the most frequently prescribed antibiotics in Gram-negative bacilli infection; and due to their use, by selective pressure, strains producing Extended-Spectrum Beta-lactamase (ESBL) are now preponderant. In a Croatian university hospital, Matanovic *et al. (*2010) put a restriction on high usage of AMC in order to reduce the high rates of

resistance in Enterobacteriaceae, particularly *E.coli*. The intervention resulted in significant decline of *E.coli* resistance rate of 37% to 11% within 18-months.

High rate of resistance was also observed against other non beta-lactam antibiotics such as tetracycline, cotrimoxazole and gentamicin among the isolates. Most of the isolates are multidrug-resistant strains, being resistant to three or more different classes of antibiotics. Ofloxacin and chloramphenicol tested against Gram-negative and Gram-positive bacteria respectively were the best drugs based on the in-vitro activity obtained. The remarkable activity of ofloxacin in this study agrees with earlier reports that *Pseudomonas aeruginosa* and *S. aureus* are highly susceptible to ofloxacin (Bakare *et al.,* 1999; Odelola and Idowu, 2007). Ofloxacin was the only antibiotic to which all the strains of *P. aeruginosa* tested were uniformly sensitive in this study.

Each of the brands of AMC was inactive against the twenty-seven out of the 28 clinical isolates (94.6%). The four brands of AMC gave similar MIC against SU11 the only strain that was sensitive to all the four brands; MIC value for AUG was twice that of the other brands. Therefore, variation in the brands appears to reflect no significance in the activity of each brand. This same result corroborates data from disk diffusion method where all the bacteria showed absolute resistance to augmentin. Majority of the Gram-negative isolates had higher MICs compared to Gram-positives. Differences in the cell envelope of the two groups are an important factor that confers inherent resistance to the Gram-negative bacteria, particularly *P aeruginosa*.

The curing of antibiotic resistance in the six bacterial isolates after exposure to mutagens at 200 µg/ml especially as evident in their sensitivity to 30 µg/ml, 12 µg/ml and 10 µg/ml supports an R-plasmid mediated resistance (Richmond and Sykes, 1987; Darini, 1996).

## Conclusion

The MIC obtained for the brands of AMC revealed that brand variation has no significance influence on its antibacterial activity. We suggest therefore that preferential prescription of a particular brand/generic form of AMC should be avoided, in support of Petra *et al*. (2007). Generic drugs should be recommended to assist patients that may not afford the expensive branded products. As much as possible indiscriminate use and consumption of drug should be totally eradicated in order to reduce the occurrence of antibiotic resistance to the barest minimum.

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