Contents lists available at www.innovativejournal.in

INNOVATIVE JOURNAL OF MEDICAL AND HEALTH SCIENCE

Journal homepage:http://www.innovativejournal.in/index.php/ijmhs

ISSN No. 2277-4939

EVALUATION OF EFFECTIVENESS OF VARIOUS COMBINATIONS OF PENICILLIN GROUPS COMMONLY USED IN NIGERIA CLINICS ON SELECTED MICROORGANISMS.

[1]Akeem Agboke*,[2]Anthony Attama,[1]Chinyere Qkoye and[1]Clement Jackson

[1]Department of Pharmaceutics and PharmaceuticalTechnology, Faculty of Pharmacy, University of Uyo, AkwaIbomState Nigeria.

[2]Department of Pharmaceutics, Faculty of Pharmaceutical SCS.University of Nigeria Nsukka,

ARTICLE INFO

Corresponding Author:
AkeemAgboke
[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, AkwaIbom State Nigeria.

Key words: Antimicrobial, activities, Combined, Penicillin, Drugs, inhibitory, Bactericidal, Concentration, spectrum.

ABSTRACT

The antimicrobial activities of different combined penicillin drugs commonly used in Nigeria were studied with the aim of establishing the minimum inhibitory concentrations of the antibiotics and their effectiveness in the treatment of some diseases caused by infectious Microorganisms, using Agar dilution method. Various standard strains of Gram +ve and Gram –ve bacteria were challenged with the antibiotics. This was carried out aseptically with varying concentrations of the antibiotics using Agar dilution method. The bacterial strains used were *Eschericia coli* (NCTC 10418), *Bacillus subtilis* (NCTC 6571), *Pseudomonas aeruginosa*(ATCC 27853), and Staphylococcus aureus (NCTC 8853). Six (6) combined penicillin group of drugs commonly used in Nigeria Clinics were used (Ampicillin + cloxacillin), (Amoxicillin + clavulanic acid), (Ampicillin + flucloxacillin), (Amoxicillin + flucloxacillin), (Ampicillin + sulbactam) and (Pipreracillin + Tazobactam). Five (5) of the Penicillin groups had activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Eschericia coli* and had no activity against *Pseudomonas aeruginosa, While only* the (Piperacillin + Tazobactam) combination had the widest antimicrobial spectrum with activity against the four test organisms and gave the lowest Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) as *B. subtilis* (MBC 0.00194 mg/ml and MIC 0.00097mg/ml), *E. coli* (MBC 0.00194 mg/ml and MIC 0.00097mg/ml), *S. aureus* (MBC 0.0312mg/ml and MIC 0.0156mg/ml), the lower the MIC and MBC, the more active the antibiotic. The statistical data analysis using ANOVA revealed that *Staphylococcus aureus* was the most sensitive of the test organisms while *Pseudomonas aeruginosa*was the most resistant to the combined penicillin drugs.

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi or protozoan (Reading and Cole,1977). An antimicrobial drug either kills microbes (bactericidal agents) or prevents the growth of microbes (bacteriostatic agents).

The *in vitro* evaluation of an antimicrobial activity of an agent means to asses its antimicrobial activity outside a living system. *In vitro* evaluation is a biological phenomena made to occur outside a living body, traditionally in a test-tube in the laboratory, (Churchill Living Stone, 1987). Thus, *in vitro* evaluation is very important because it can be used to predict the *in vivo* (inside the body) activity of the given antimicrobial agent (Akunyili and Akubue, 1995).Technically, antibiotics are only those substances that are produced by one micro-organism that kills or prevents the growth of another micro-organism (Okore, 2005). In today's common usage, the term antibiotic is used to refer to almost any substancethat attempt to rid the body of bacterial infections. Antimicrobial includes not just antibiotics, but synthetically formed compounds as well.

Resistances which have been developed by microorganisms affect different groups of penicillins (Lippincott, 2009). Benzylpenicllin and phenoxymethylpenicillin are active against susceptible strains of Gram positive and Gram-negative bacteria, spirochetes and actinomycetes; but are inactivated by penicillinase and other beta-lactamases enzyme producing bacteria. Benzathinebenzylpenicillin and procaine benzylpenicillin are long acting preparations which slowly release benzylpenicillin on injection. A range of penicillin with improved stability to gastric acid and penicillinase has been produced by substitution of 6-amino position of 6-amino penicillinic acid (EMDEX, 2008/09). Beta-lactam antibiotics like penicillin, cephamycins, and carbapenems, although carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by

breaking the antibiotics' structure (Woodford N, Ward E, Kaufmann ME, *et al, 2006*). These antibiotics all have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open, deactivating the molecule's antibacterial properties(Santillana E, Beceiro A, Bou G, Romero A, 2007).Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria.

Penicillinase is a specific type of β-lactamase, showing specificity for penicillins. It was the first β-lactamase to be identified and was first isolated by Abraham and Chain in 1940 from Gram-negative *E. coli* even before penicillin entered clinical use, but penicillinase production quickly spread to bacteria that previously did not produce it or produced it only rarely (Hall BG, Barlow M, 2004)). Penicillinase-resistant beta-lactams such as methicillin were developed, but there is now widespread resistance to even these (Hall BG, Salipante SJ, Barlow M, 2004).

The MIC (Minimal Inhibitory Concentration) of a bacterium to a certain antimicrobial agent can be determined and today gives the best quantitative estimate for susceptibility(Gaudreau C, et al.2008). MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the bacteria. The principle is simple: Agar plates, tubes or micro-titre trays with two-fold dilutions of the antibiotics are inoculated with the bacteria and incubated 37^{o}C for 24 to 48 hours then observed for inhibition or growth of the organisms (CLSI.2009).The MIC tells you about the degree of resistance and might give you important information about the resistance mechanism and the resistance genes involved. MIC-determination performed as agar dilution is regarded as the golden standard for susceptibility testing (Gaudreau C, et al**.** 2007). In contrast, diffusion tests are primarily qualitative methods that normally should only be used to report whether a bacterium is resistant or not. The principle involves an agar plate after inoculation with the bacteria, a tablet, disk or paper strip with antimicrobial agent is placed on the surface. During incubation the antimicrobial agent diffuses into the agar and inhibits growth of the bacteria if sensitive. Diffusion tests are cheap compared to most MIC-determination methods. E-test is a diffusion test, but has been developed to give an approximate MIC-value **(**Gaudreau C, et al.2007).

Well standardized methods are essential for all kinds of susceptibility testing, since the methods are highly sensitive to variations in several factors, for example, size of inoculum, contents and acidity of the growth medium, time and temperature of incubation. The agar diffusion methods are also strongly influenced by factors such as agar depth, diffusion rate of the antimicrobial agent and growth rate of the specific bacteria (Okore, V C (2005).

The objective of this research work is to evaluate the antimicrobial activity of different generic combination of penicillin antibiotics and to identify the most effective combination of the penicillin antibiotics.

MATERIALS AND METHODS

Culture Media

These were used as supplied from the manufacturers after being subjected to sterilization. These include Nutrient Agar (Oxoid Ltd) , Mueller Hinton Agar (Oxoid Ltd, Basingstokes Hampshire, England) and Nutrient broth. The media were prepared according to manufacturer's specification.

Antimicrobial Test Organisms

The following typed bacterial insolates were used,*Eschericia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6571), *Pseucbmonasaeruginosa* (ATCC 27853), *Bacillus subtilis* (NCTC 8853). These organisms were standard isolates obtained from the pharmaceutical microbiology laboratory, Faculty of pharmacy, University of Lagos, Nigeria. They were sub-cultured in solid medium (Nutrient Agar) and incubated at 37^{o}c. One colony aseptically transferred into 20ml Nutrient broth to have over night broth culture.

Drugs Used

Table 1.0 Generic drug in Combination

Generic Combination	Brand Name	Manufacturer
Amoxicillin sodium and clavulanate potassium for injection BP.	Cerobact 1.2g	Zhuhai United Laborotaories (zohongshon) Co. Ltd.
Ampicillin and cloxacillin	Ampiclox 500mg	Beecham.
Ampicillin and flucloxacillin	Cofluampicil 500mg	Beecham and Nichben Pharm. Ltd.
Amoxicillin and flucloxcillin	Espapen 500mg	MedreichPlc
Ampicillin and sulbactam	Unasyn 1.5g	Pfizer
Piperacillin and Tazobactam for injection	Zosyn	Wyeth pharmaceutical limited

METHODS

Preparation of the Sample Concentration of the Antibiotics

100mg each of the drug samples was aseptically transferred into sterile 100ml conical flak. Each of the conical flasks was properly labeled and the samples measured were dissolved with 100ml of sterile water to obtain a stock concentration of 1mg/ml of each of the drug sample.

Sterilization of Apparatus

Glasswares were sterilized in hot air-over (Gen. Lab) at 160^{o}c$\pm$ 5^{o}c for 1 hour. Plastic containers and working benches were cleaned with 70% alcohol. Microbiological media and preparation bottles, test-tubes and pipettes were sterilized in an autoclave at 121^{o}c for 15minutes at 151b/sq inch.

Preparation of Culture

The standard isolate of the bacterial were subcultured on Nutrient Agar. These were further incubated at 37^{o}c for 24 hours.

Sensitivity Test

A stock solution of the drug samples were prepared by dissolving 100mg drug samples powder in 100ml of water. The solutions obtained were further diluted to give varying concentrations.

The same concentrations of cerobact injection, Ampiclox capsule, cofluampicil capsule, Espapen capsule, Unasyn injection and Zosyn injection were used respectively for testing the antibacterial activity. Varying concentrations of these were obtained respectively using twofold serial dilutions.

Agar plate diffusion method was used for the sensitivity test. 24 sterile disposable Petri dishes were used. A range of 3–5g of Muller Hinton Agar was dissolved in 100ml sterile distilled water and sterilized by autoclaving at 121^{o}c for 15 minutes and allowed to cool to 40–45^{o}c. Suspension of the organisms each in 0.02ml

aliquot, measured with a micropipette were used as test organisms. The organism used for the test were *Eschericia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6571), *Pseudomonasaeruginosa* (ATCC 27853), and *BacilliusSubtilus* (NCTC 8853). A 25ml sample of molten Muller Hinton Agar was poured into the Petri-dishes and proper mixing was ensured before solidification.

Sterile cork borer with a diameter of 4mm was used to bore 4 holes in each of the plate used for the sensitivity test. By means of a micropipette (Oxford) varying concentration of Cerobact injection, Ampiclox capsule, Confluampicil capsule, Espapen capsule, Unasyn injection and Zosyn injection were filled to the brim of the hole made on the agar medium with the aid of micropipette (Oxford).

These preparations were then incubated at 37^{o}c for 48 hours for anti bacterial activity. The different zones of inhibition were then measured using a metric rule. The diameter of the clear spot i.e. area free of any growth of organisms was noted and the diameter of the cork borer was subtracted from this to give the zone of inhibition.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using tube-dilution method. A total number of 172 sterile test-tubes were used. 25mg of Nutrient broth was dissolved in a 1000ml of sterile distilled water and sterilized by autoclaving at 121^{o}c for 15 minutes and allowed to cool to 40 – 45^{o}c. The empty sterile test- tubes were properly labeled and 5ml of the sterile nutrient broth was aseptically transferred into each of the test-tube. A two fold serial dilution of the drug sample were aseptically carried out using the least concentration for sensitivity for each of the drug samples respectively as the stock, to obtain varying concentration of the drug samples. Each of the dilution of the drug samples were inoculated with 0.02ml of the test culture measured with micropipette. These test-tubes were incubated at 37^{o}c for 48 hours. The tubes were examined visually for presence of growth indicated by the turbidity of the test-tubes. The tube with the lowest concentration of drug samples inhibiting growth gives the MIC.

The minimum inhibitory concentration (MIC) tubes without growth were then selected, an aliquot of inocula from these non-turbid minimum inhibitory concentration (MIC) tubes were then subcultured on a solid nutrient agar plate at 37^{o}c for 48 hours. The lowest concentration of each drug samples against each test organism that allowed less than 0.1% of the logically inoculum to survive gives minimum bacterial concentration, (Okore, 2005).

Statistical data analysis

To observe if there was significant difference in activity among the drugs, data were plotted into SPSS 17 software and ANOVA test performed.

RESULTS

RESULT OF ANTI-BACTERIAL SENSITIVITY TEST

Table 1.1: Zones of inhibition (mm) of Ampicillin + Cloxacillin combination using *Bacillus Subtilis, Staphylococcus aureus,Peudomonasaeruginosa* and *Eschericia coli* as organisms.

Drug Concentration	Zones of inhibition (mm)			
Ampiclox + cloxacillin (mg/ml)	*B. Subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
1.0	14.0mm	Complete inhibition	No inhibition	17.0
0.5	12.0	Complete inhibition	No inhibition	16.0
0.25	8.0	Complete inhibition	No inhibition	15.0
0.125	7.0	Complete inhibition	No inhibition	14.20

Table 1.2: Zones of inhibition (mm) of Amoxicillin + Clavulanic acid combination using *Bacillus Subtilis, Staphylococcus aureus,Pseudomonas aeruginosa* and *Eschericia coli* as test organism.

Drug Concentration	Zones of inhibition (mm)			
Amoxicillin + Clavulanic acid (mg/ml)	*B.subtilis*	*S.aureus*	*P.aeruginosa*	*E. coli*
1.0	14.5	Complete inhibition	No inhibition	26.0
0.5	12.0	Complete inhibition	No inhibition	23.0
0.25	11.0	Complete inhibition	No inhibition	20.0
0.125	10.0	Complete inhibition	No inhibition	15.0

Table 1.3: Zones of inhibition (mm) of Ampicillin + flucloxacillin combination using *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Eschericia coli* as test organisms.

Drug concentrations	Zones of inhibition (mm)			
Amoxicillin + flucloxacillin (mg/ml)	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
1.0	13.0	Complete inhibition	No inhibition	20.0
0.5	12.0	Complete inhibition	No inhibition	17.0
0.25	10.0	Complete inhibition	No inhibition	15.0
0.125	9.0	Complete inhibition	No inhibition	10.0

Table 1.4: Zones of inhibition (mm) of Amoxicillin + flucloxacillin combination using *Bacillus subtilis,Staphylococcus aureus, Pseudomonas aeruginosa* and *Eschericia coli* as test organisms.

Drug concentrations	Zones of inhibition (mm)			
Amoxicillin + flucloxacillin (mg/ml)	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
1.0	13.0	Complete inhibition	No inhibition	18.0
0.5	11.0	Complete inhibition	No inhibition	15.0
0.25	8.0	Complete inhibition	No inhibition	14.0
0.125	5.0	Complete inhibition	No inhibition	10.0

Table 1.5: Zones of inhibition (mm) of Amoxicillin + flucloxacillin combination using *Bacillus subtilis,Staphylococcus aureus, Pseudomonasaeruginosa* and *Eschericia coli* as test organisms.

Drug concentration	Zones of inhibition (mm)			
Ampicillin + Sulbactam (mg/ml)	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*
1.0	14. 0	Complete inhibition	No inhibition	22.0
0.5	12.0	Complete inhibition	No inhibition	20.0
0.25	10.0	Complete inhibition	No inhibition	17.0
0.125	8.0	Complete inhibition	No inhibition	14.0

Table 1.6: zones of inhibition (mm) of piperacillin + tazobactam combination using *bacillus subtilis staphylococcus aureus, pseudomonas aeruginosa* and *Eschericia coli* as test organisms

Drug concentration	Zones of inhibition (mm)			
Piperacillin + Tazobactam	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*

(mg/ml)				
1.0	26.5	Complete inhibition	26.0	16.0
0.5	22.0	Complete inhibition	20.0	14.0
0.25	20.0	Complete inhibition	18.0	12.0
0.125	16.0	Complete inhibition	16.0	10.0

TABLE 1.7: Zones of inhibition (mm) of all the drug samples at lower concentration using *staphylococeusaureus* at a test organism (this is because at a higher concentration, it gives complete inhibition).

Drug concentration	Zones of inhibition (mm)					
	Ampicillin + Cloxacillin	Amoxicillin + Clavulanate	Ampicillin + Flucloxacillin	Amoxicillin + Flucloxacillin	Ampicillin + Sulbactam	Piperacillin + Tazobactam
0.0625	34.0	28.0	33.0	31.0	24.0	31.0
0.0312	29.0	26.0	28.0	27.0	20.0	27.0
0.015625	24.0	23.0	26.0	25.0	18.0	24.0
0.0078125	22.0	21.0	25.0	22.0	16.0	21.0

N/B: All the results were recorded after subtraction of 4mm of the hole made by the cork borer.

RESULT OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF DRUG SAMPLES AGAINST THE TEST ORGANISMS

Table 1.8: MIC and MBC result of Ampicillin + Cloxacillin combination using *Bacillus subtilis, Eschericia coli, Staphylococcus aureus.*

Drug concentration	MIC and MBC Result			
Ampicillin + cloxacillin (mg/ml	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-	-	-	+
0.03125	-	-	-	+
0.015625	-*	-	-	+
0.0078125	-.	-*	-	+
0.00390625	+	-.	-*	+
0.001953125	+	+	-.	+
0.000976525	+	+	+	+
0.00048328125	+	+	+	+

Table 1.9: MIC and MBC result of Ampicillin and Clavulanic acid combination acid using *Bacillus subtilis, Eshericia coli*, and *Staphylococcus aureus*.

Drug concentration	MIC and MBC Result			
Ampicillin + Clavulanic acid (mg/ml)	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-	-	-	+
0.03125	-	-	-	+
0.015625	-*	-*	-	+
0.0078125	-.	-.	-	+
0.00390625	+	+	-*	+
0.001953125	+	+	-.	+
0.000976525	+	+	+	+
0.00048328125	+	+	+	+

Table 2.0: MIC and MBC result of Amplicillin + flucloxacllin using *Bacillus subtilis, Eschericia coli* and *Staphylococcus aureus.*

Drug concentration	MIC and MBC Result			
Amplicillin + flucloxacllin(mg/ml)	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-*	-	-	+
0.03125	-.	-	-	+
0.015625	+	-	-	+
0.0078125	+	-*	-	+
0.00390625	+	-.	-	+
0.001953125	+	+	-*	+
0.000976525	+	+	-.	+
0.00048328125	+	+	+	+

Table 2.1:MIC and MBC result of Amoxicillin + flucloxacillin combination using *Bacillus subtiles, Eschericia coli* and *staphylococcus aureus*

Drug concentration	MIC and MBC Result			
Amoxicillin + flucloxacillin (mg/ml)	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-	-	-	+
0.03125	-	-*	-	+
0.015625	-*	-,	-	+
0.0078125	-.	+	-*	+
0.00390625	+	+	-.	+
0.001953125	+	+	+	+
0.000976525	+	+	+	+
0.00048328125	+	+	+	+

Table 2.2:MIC and MBC result of Ampicillin + sulbactam combination using *Bacillus subtilis, Eschericia coli* and *staphylococcus aureus*

Drug concentration	MIC and MBC Result			
Ampicillin + sulbatam (mg/ml)	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-	-	-	+
0.03125	-	-	-	+
0.015625	-	-	-	+
0.015625	-	-	-	+
0.0078125	-	-	-	+
0.00390625	-	-*	-	+
0.001953125	-*	-*	-*	+
0.000976525	-*	+	-*	+
0.00048328125	+	+	+	+

Table 2.3: MIC and MBC result of Piperacillin + Tazobactam combination using *Baccillussubtilis, Eschericia coli, staphylococcus aureaus* and *pseudomonas aeruginosa*

Drug concentration	MIC and MBC Result			
Piperacillin + Tazobactam (mg/ml)	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
0.0625	-	-	-	-
0.03125	-	-	-*	-*
0.015625	-	-	-*	-*
0.015625	-	-	+	+
0.0078125	-	-	+	+
0.00390625	-	-	+	+
0.001953125	-*	-*	+	+
0.000976525	-*	-*	+	+
0.00048328125	+	+	+	+

Key

-	=	*No growth*
+	=	*Growth*
*	=	*MIC (Minimum Inhibitory Concentration)*
*	=	*MBC (Minimum Bactericidal Concentration).*

DISCUSSION

In Table 1.1:Ampicillin + Cloxacillinhad activity against the organisms with their various Zone of inhibitions Bacillus subtilis (7mm – 14mm), *Eschericia coli* (14.2mm – 17.0mm) *Staphylococcus aureus* (22mm – 34mm), no activity against *Pseudomonas aerruginosa*. As shown in the table.

Table 1.2: Amoxicillin + Clavulanic acid showed activity against the organismswith zone of inhibitions as follows; *Bacillus subtilus*(10.0mm – 14.5mm), *Eschericia coli* (15.0mm – 26.0mm), *Staphylococcus aureus* (21.0 – 28.0mm), but no activity against *Pseudomonas aeruginosa*.

Table 1.3: Ampicillin + Flucloxicillinshowed activity against *Bacillus subtilis* with a zone of inhibition (9.mm – 13.0mm) *Eschericia coli* (10.0mm – 20.0mm),

Staphylococcus aureus (25.0mm – 33.0mm). It had no activity against *Pseudomonas aeruginosa*.

Table 1.4: Amoxicillin + Flucloxicillinhad activity against *Bacillus subtilis* (5.0mm – 13.0mm), *Eschericia coli* (10.0mm – 18.2mm), *Staphylococcus aureus*(22.0mm – 31.0mm),no activity against *Pseudomonas aeruginosa*

Table 1.5: Ampicillin + Sulbactamshowed activity against *Bacillus subtilis* with a zone of inhibition between (8.0mm – 14.0mm), *Eschericia coli* (14.0 – 22.0mm), *Staphylococcusaureus*(16.0mm – 24mm). It had no activity against *Pseudomonas aeruginosa.*

Table 1.6: Piperacillin + Tazobactamhad activity against the four test organisms; *Bacillus subtilis* (16.0mm – 26.5mm), *Eschericia coli* (10.0mm – 16.0mm), *Staphylococcus aureus* (21.0mm – 31.0mm), *Pseudomonas aeruginosa* (16.0mm – 26.0mm)

Table 1.7: At concentration of 0.125mg/ml – 1.0mg/ml, *Staphylococcus aureus* showed no growth (i.e. complete inhibition) for all the combined penicillin drugs, but at a reduced concentration of 0.0078125mg/ml-0.0625mg/ml, following two fold serial dilution, *Staphylococcus aureus* showed tremendous zones of inhibition with all the drugs. This indicates that *Staphylococcus aureus* is highly susceptible to all the combined penicillin drugs, especially with beta lactamase inhibition (Lippincott, 2009), as shown in the table.

Table 1.8–2.3:

At concentrations of 0.125mg/ml-1.0mg/ml, *Pseudomonas aeruginosa* showed no inhibition with all the combined penicillin drugs used except Piperacilin + Tozabactam combination. This shows piperacillin combination is the most potent combined penicillin drug and this as gone a long way in overcoming the problem of serious infections caused by *Pseudomonas aeruginosa*and as such they are also known as penicillins that have anti pseudomonal activity (Lippincott, 2009).

The results of the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of antibiotic against the different test organisms shows that Piperacillin + Tazobactam combination had a wider spectrum of activity than others. It had the MIC of 0.001953125mg/ml against *Bacillus subtilis, Eschericia coli, Staphylococcus aureus*and 0.03125mg/ml against *Pseudomonas aeruginosa*respectively. It also had minimum bacterial concentration of 0.000976525mg/ml against *Bacillus subtilis, Eschericia coli, Staphylococcus aureus*and 0.015625mg/ml against *Pseudomonas aeruginosa respectively*.

Statistical analysis using Anova on SPSS Version 17 software revealed that the significant differences within the antibiotics combination vary from (P< 0.0001- 0.01).

CONCLUSION

Piperacillin + Tazobactam combination had the widest spectrum of activity, as it is effective against the four test pathogenic organisms and also the most potent of the entire antibiotic used, because it gives the lowest Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) for all the organisms.

Others (Ampicillin + Cloxacillin) combination, (Amoxicillin +Clavulanic acid) combination, (Ampicillin + Flucloxacillin) combination, (Amoxicillin + Flucloxacillin) combination, (Ampicillin + Sulbactam) combination, had antibacterial activity against *Bacillus subtilis, Eschericia coli,* and *Staphylococcus aureus* but had no activity against *Pseudomonas aeruginosa*.

The administration of these penicillin combination drugs to infectious patient is an established clinical method of treating infections that are not responding to single penicillin drug. However proper diagnosis and laboratory sensitivity test should be done before considering the choice of penicillin combination drug to be used.

It is concluded from this research work that among the commonly used penicillin combined drugs in Nigeria, Piperacillin + Tazobactam (Zosyn) combination had the widest spectrum of activity, as it is effective against all the organisms used including *Pseudomonas aeruginosa*.

REFERENCES

1. Barlow M, Hall BG (2002) Phylogenetic analysis shows that the OXA beta-lactamase genes have been on plasmids for millions of years. J MolEvol 55(3):314-321.
2. CLSI**.** 2009. Methods for dilution antimicrobial susceptibility test forbacteria that grow aerobically; approved standard— 8th edition. CLSIdocument M07–A8. Clinical and Laboratory Standards Institute,Wayne, PA.
3. Cuffini, A. M, Tullio, V, LanniParachio, A. et al (1998). Enhanced effect Amoxycillinclavulanate compared with amoxicillin and clavulanate on the susceptibility to immunodefenses of penicillin-resistance strain. Drugs under experimental and clinical research 24, 173 – 84.
4. EMDEX, Based on who mode formulary (2009). The complete drug formulary for Nigeria's Health Professionals 2008/09 Edition. LINDOZ Books INT'L, Canada. PP 279 – 97.
5. Gaudreau C, et al**.** 2008. Comparison of disk diffusion and agar dilution methods for erythromycin, ciprofloxacin, and tetracycline susceptibility testing of *Campylobacter coli* and for tetracycline susceptibility testing of *Campylobacter jejuni*subsp. *jejuni*. Antimicrob. Agents Chemother. **52**:4475–4477.
6. Gaudreau C, et al**.** 2007. Comparison of disk diffusion and agar dilution methods for erythromycin and ciprofloxacin susceptibility testing of *Campylobacter jejuni*subsp. *jejuni*. Antimicrob. Agents Chemother. **51**:1524–1526.
7. Hall BG, Salipante SJ, Barlow M (2004) Independent origins of subgroup Bl + B2 and subgroup B3 metallo-beta-lactamases. J MolEvol 59(1):133-141
8. Hall BG, Barlow M (2003) Structure-based phylogenies of the serine beta-lactamases. J MolEvol 57(3):255-260
9. Hall BG, Barlow M (2004) Evolution of the serine beta-lactamases: past, present and future. Drug Resist Updat 7(2):111-223.
10. Lippincolt, W. and Lippincott, W (2007). Pharmacology 4th edition. Welter Kluwer, New York. Pp 359 – 64.
11. Nordmann P, Cuzon G, Naas T (April 2009). "The real threat of Klebsiellapneumoniaecarbapenemase-producing bacteria".*Lancet Infect Dis*9 (4): 228–36. doi:10.1016/S1473-3099(09)70054-4. PMID 19324295.
12. Okore, V C (2005) Pharmaceutical Microbiology. ELDEMAK, Enugu pp. 181 – 194.
13. O'Callaghan CH, Morris A, Kirby SM, Shingler AH (April 1972). "Novel method for detection of beta-lactamases by using a chromogenic cephalosporin substrate".

*Antimicrob. Agents Chemother.*1 (4): 283–8. doi:10.1128/AAC.1.4.283. PMC 444209. PMID 4208895.

14. Reading, C. and Cole, M. (1977). Calvulanate: a beta-lactamase-inhibiting beta-lactam from *streptomycesclavuligerus*. Antimicrobial Agents and Chemotherapy 11, 852 – 7.
15. Santillana E, Beceiro A, Bou G, Romero A (March 2007). "Crystal structure of the carbapenemase OXA-24 reveals insights into the mechanism of carbapenem hydrolysis". *Proc. Natl. Acad. Sci. U.S.A.*104 (13): 5354–9. doi:10.1073/pnas.0607557104. PMC 1838445. PMID 17374723
16. Woodford N, Ward E, Kaufmann ME, *et al*.."Molecular characterisation of *Escherichia coli* isolates producing CTX-M-15 extended-spectrum β-lactamase (ESBL) in the United Kingdom" (PDF). Health Protection Agency. Retrieved 2006-11-19.
17. Walsh TR, Weeks J, Livermore DM, Toleman MA (May 2011). "Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study". *Lancet Infect Dis*11 (5): 355–62. doi:10.1016/S1473-3099(11)70059-7. PMID 21478057.