Bacterial resistance to cephalosporin in clinical isolates in Jos University Teaching Hospital (JUTH)

Vivian Okemena Agbor, Lynn Ma'ori

Department of Medical Microbiology, Federal School of Medical laboratory Sciences Jos, Plateau State, P. M. B 2253, Nigeria. lynnmaori09@gmail.com

Abstract: Bacterial resistance to cephalosporin in clinical isolates in Jos University Teaching Hospital (JUTH) was carried out between November and April, 2011. 60 bacterial isolates were collected from Urine, Blood, Stool, Wound, Ear, Throat and High Vaginal Swabs. Cultural and biochemical techniques were used to identify the isolates. Out of the 60 isolates, 26(43.3%) were resistant to the prepared first generation (Cephalexin), 5(8.3%) to 2nd generation (Cefuroxime), 4(6.6%) to 3rd generation and 9(15.0%) were resistant to control used (commercially prepared Ceftazidime 30ug). *Salmonella spp* were shown to be susceptible to all the generation of Cephalosporins with 0(0%) resistance while *Pseudomonas spp* were shown to be resistant to all the generations of Cephalosporins with 9(100%), 6(66.6%), 1(11.1%) to 1st, 2nd, and 3rd generations respectively and control with 1(11%) which is also a second generation of Cephalosporins. Therefore, even among the 2nd generations some are more active than others while 3rd generation of Cephalosporins are still more effective over the others from the result of this study.
[Vivian Okemena Agbor[1], Lynn Ma'ori. **Bacterial resistance to cephalosporin in clinical isolates in Jos University Teaching Hospital (JUTH).]** New York Science Journal 2011;4(9):46-55]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork.

1. Introduction

An antibiotic (from the ancient Greek Anti "against" and bios "life") is a substance or compound that kills or inhibits the growth of bacteria (Dorlands medical Dictionary, 2010). Those that kill bacteria are bactericidal while those that inhibits the growth of organisms are bacteriostatics (Davey, 2000). Antibiotics were coined by Selwan Waksman in 1942 to describe any substance produced by a micro-organism that is antagonistic to the growth of other micro-organisms in high dilution (Waksman, 1947).

These antibiotics can be natural or synthetic. Antibiotics are one class of antimicrobials a larger group which also includes anti-viral, anti-fungal and anti-parasitic drugs. The term coined by Selman Waksman excluded naturally occurring substances that kill bacteria but are not produced by micro-organisms (such as gastric juice and hydrogen peroxide) and also exclude synthetic antibacterial compounds such as the Sulphonamides with a molecular weight less than 2000 atomic mass unit (Von Nussabaum, *et al*, 2006). Commonly used antibiotics include the *Penicillins, Cephalosporins, Aminoglycosides, Chloramplenicol, Tetracyclines, Polymyxins, Eythromycins* etc and the common synthetic antimicrobials are the *Sulphonamides, Trimethoprin, Nalidixic* acid etc (Ochei, *et al*., 2007). Antibiotics are commonly classified based on their mechanisms of action, chemical structure or spectrum of activity. Most of them target bacterial function or growth process (Calderon, *et al*., 2007). Those that target the bacterial cell wall (Penicillin, Cephalosporins) or cell membrane (Polymixins) or

interfere with essential bacterial enzymes (Quinolones, Sulphonamides) are usually bacteriacidal in nature. Those that target protein synthesis such as Aminoglycocides, Tetracyclines, Chloramphenicol are usually bacteriostatic (Finberg, *et al*., 2004).

Antibiotics can be categorized based on their target specificity "narrow spectrum" antibiotics target particularly types of bacterial such as gram negative (*Enterobacteria*) or gram positive bacteria (*Staphylococci*). While "wide spectrum" antibiotics affect a larger range of bacteria (Slama *et al*., 2005). The Cephalosporins (i.e. first generation) were discovered to combact the problems of betalactamase production. Findings later discovered that many bacterial were able to developed resistance to them. This prompted the introduction of the second generation Cephalosporins i.e. which were able to correct the deficiency of the previous class. But the relief from this discovery was short lived as emergence of resistance was soon observed. Now it is not only the third generation but fourth generation of Cephalosporin that are on the market.

Presently, Ceftriaxone a third generation Cephalosporin is the drug of choice to treat infantile Salmonellosis due to the contradiction of the quinolones in this class of patients. But report has shown emergence of resistance of Salmonella to this agent. This is a sad story not only for Salmonellosis but other life threatening infections.

Unless antibiotics resistance problems are detected as they emerge and actions taken immediately to contain them, society could be faced

with previously treatable diseases that have become again untreatable as in the days before antibiotics were developed (Sheldon, 2005). We must remain vigilant regarding the rational use of these extremely valuable clinical agents to assure their continuance efficiency. This study determined the degree of bacteria resistance to the different generation of Cephalosporins in JUTH (Jos University Teaching Hospital) and found the most effective class of Cephalosporins to the clinical isolates in JUTH and determined the group of bacterial isolates that are most resistant to the Cephalosporins. After the discovery of the first antibiotics Penicillin by Alexander Fleming (1928), more antibiotics became commercially available. Although these new antibiotics were looked as "wonder drugs" initially, the resistant bacterial strains soon started emerging and susceptibility tests for these drugs became necessary (Ochei, *et al*. 2007).

Broad spectrum drugs have an advance effect on the normal floral that have protective good in the body such as the mouth and the vagina which narrow spectrum drugs do not have, therefore, narrow spectrum drugs are preferable for the treatment of known pathogens (Ochei, *et al*., 2007).

Antimicrobial drugs act on one of several ways: by selective toxicity whereby the agents is harmful to a pathogen without being harmful to the host (Jawetz, *et al.,* 2010) some of the antibiotic use for this selective toxicity are Penicillin, Chloramphenicol Cephalosporins, Tetracycline etc.

MECHANISMS OF ACTION OF ANTIBIOTICS
The mechanisms of action of antibiotics are classified based on their activity on the bacteria:
- Inhibition of cell wall synthesis, e.g. Penicillin and Cephalosporins.
- Inhibition of cell membrane function e.g. Polymixins
- Inhibition of protein synthesis e.g. Aminoglycosides, Chloramphenicol, Gentamycin etc.
- Inhibition of Nucleic acid synthesis e.g. Rifampicin
- Inhibition of Folic acid synthesis e.g. Sulphonamides.

Inhibition Of Cell Membrane Synthesis
The cytoplasm of all living cells is bounded by the cytoplasmic membrane which serves as a selective permeability barrier, carries out active transport functions and thus contrast the internal composition of the cell. Some of these antibiotics act as cationic detergents and bind to the cell membrane. This binding results in the loss of semi-permeability of the membrane, leading to the loss of cell contents and cell death. Detergents, which contain lipophilic and hydrophilic groups, disrupt cytoplasmic

membranes and kill the cell. Example, Polymixins, consists of detergent like cyclic peptides that selectively damage membranes containing phosphatidylethanolamine, a major component of bacteria membranes. Other antibiotics such as Nalidixic and specifically interfered with biosynthetic functions of the cytoplasmic membrane by inhibiting DNA synthesis (Jawetz, *et al.,* 2010).

Inhibition Of Protein Synthesis
Some of these antibiotics are protein synthesis by affecting the translation in the cell. Example are the Aminoglycoisdes, Tetracyclines, Erythromycins, Lincomycins, Chloramphenicol etc.

The Aminoglycosides attach to a specific receptor protein (P12 in the case of Streptomycin) on the 30s subunit of the microbial ribosome.

It also blocks the normal activity of the "initiation complex" of peptide formation (MRNA + formly methionine + tRNA) (Jawetz, *et al*., 2010).

Inhibition Of Folic Acid Synthesis
Folic acid is needed as a cofactor in the synthesis of thymidine and other Nucleotides. Sulphonamide resembles Para-aminobenzoic acid (PABA) and can enter into folic acid synthesis in place of PABA. As a result, non-functional analogs of folic acid are formed preventing the synthesis of thymidine which is an essential ingredient of nuclide acid. Therefore further growth of bacteria is thus arrested e.g. Sulphonamide (Ochei, *et al*., 2007).

Inhibition Of Nucleic Acid Synthesis
Antibiotics inhibit nucleic acid synthesis of the organism there by preventing multiplication of the bacteria. These antibiotics such as Rifampin inhibit bacterial growth by binding strongly to the DNA dependant RNA polymerase of bacteria. Thus it inhibits bacterial RNA polymerase due to a chromosomal mutation that occurs with high frequency (Jawetz, *et al*., 2010).

Inhibition Of Cell Wall Synthesis
Bacterial have rigid outer layer, the cell wall. The cell wall maintains the shape and size of the microorganisms which has a high internal osmotic pressure. Inhibition of its formation may lead to lysis of the cell wall by preventing the cross linking of the polysaccharide chains in the polypeptidoglycan layer of the cell wall. Example are Penicillin, Cephalosporins, Vacomycin etc (Ochei, *et al*; 2007). Cephalosporins, an inhibitor of cell wall synthesis are group of antibiotics similar to Penicillin that are widely used and trustworthy antibiotics in daily practice (Chemother, 2005). Cephalosporins are derived from some Cephalosporin fungi yielded antimicrobial substances (Jawetz, *et al.,* 2010).

They are beta-lactam compound in which the beta-lactam ring is fused to a 6-membered dihydrothiazine ring thus, forming Cephalosporin

nucleus. Cephalosporin compound were first isolated from cultures of *Cephalosporin acremonium* from a sewer in sardine in 1948 by Italian scientists, Guiseppe Brotzu (Podolsky, 1998). He noticed that there cultures produced substances that were effective against. *Salmonella typhi*, the cause of typhoid fever, which has beta-lactamase. Guy Newton and Edward Abraham at the Sir William Dunn School of Pathology at the University of Oxford isolated *Cephalosporin C*. The Cephalosporin nucleus 7-amino Cephalosporinic acid (7-ACA), was derived from Cephalosporin C and proved to be analogous to the Penicillin nucleus 6-amino Penicillin Acid (6-APA), but it was not sufficiently potent for clinical use (Podolsky, *et al*, 1998).

Modification of the 7- ACA side chains resulted in the development of useful antibiotics agents and the first agent Cephalothin (cepalotin) was launched by Eli Lilly and company in 1964.

MECHANISM OF ACTION OF CEPHALOSPORINS

Natural Cephalosporins have low antimicrobial activity, but the attachment of various R side groups has resulted in the proliferation of an enormous array of drugs with varying pharmacologic properties and antimicrobial spectra and activity (Jawetz, *et al,* 2010).

Cephalosporins are bactericidal and have the same mode of action as the other beta-lactam antibiotics such as Penicillin but are less susceptible to penicillinases. The mode of action can be summarized as follows:

(i)   Disruption of the synthesis of Peptidoglycan layer of bacteria cell wall by blocking the transpeptidation of Peptidoglycan. The Peptidoglycan layer is important for cell was structural integrity. The final Transpeptidation step in the synthesis of the Peptidoglycan is facilitated by transpepticlases known as Penicillin – binging proteins (PBPs) which is an enzyme (Podolsky, 1998).

(ii) Binding of specific penicillin – binding protein (PBPs) that serve as a drug receptor on bacteria. The PBPs binds to the D-ala at the end of muropeptides (peptdoglycan precursor) to crosslink the Peptidoglycan (Podolsky, 1998).

(iii) Activating analytic enzyme in the cell wall that can produce lesions resulting in bacteria death (Jawetz, *et al.*, 2010).

The Cephalosporins nucleus can be modified to gain different properties. Based on their spectrum of activity and also for ease of reference, Cephalosporins have been arrayed into major groups and also broadly categorized into 4 (four)

generations; (first to fourth). The fourth generation Cephalosporins as at March 2007, were considered to be "a class of light potent antibiotics that are among mediciners last defences against several human infections according to the Washington post (FDA, 2009).

The first Cephalosporins were designated first generation Cephalosporins, wereas later more extended-spectrum Cephalosporins were designated as second generations and third generation Cephalosporins. Each newer generation Cephalosporins has significantly greater Gram negative antimicrobial properties that the preceeding generation, in most cases with decreased activity against Gram positive organisms. Fourth generation of Cephalosporins however, have true broad-spectrum activity (Pichichero, 2006).

The classification of Cephalosporins into generations is a common practice although the exact categorization is often imprecise. For example, the fourth generation Cephalosporin is not yet recognized in Japan where, cephalor is classified as a first generation Cephalosporins even though in the United States, it is a second generation one. Also Cephbuperazone, Cephminox and Cephatelan are classified as second generation Cephalosporins. Cephmetazole and Cephalexn are classed as third generation cephem (British National formular, 2008).

Most first generation Cephalosporins were originally spelled "ceph" in English-speaking countries. This continues to be the preferred spelling in United States and Australia, while European countries including the United Kingdom have adopted the international non-proprietary names, which are always spelled "cef". Newer, first generations Cephalosporins and all Cephalosporins of late generations are spelled "cef" even in the United States (Stock, 2006).

These are the group of the first generation Cephalosporins

Cephalothin, Cephapirin, Cetazolin, Cephalexin, Cephradine Cefadroxil

The following are members of the second generation Cephalosporins.

Cefamandole, cefuroxime, Cefonicid, Ceforamide, Cefalor, Cetoxitin, Cefotetan, Cefproxil, Cefuroxime axetil, Cefmetazole.

The 3rd generation are Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftazidime, Cefoperazone, 4th generation: Cefixime, Cefpodoxixime proxetil, Ceftibuten, Cefdinir Cefepime, Cefpirome

SPECTRUM OF ACTIVITY OF CEPHALOSPORINS

In general, first generation Cephalosporins have better activity against Gram-positive bacteria

and less Gram-negative activity while third generation agents with a few exception have better Gram-negative activity and less Gram-positive activity.

The fourth generation has both Gram-positive and Gram-negative activity (Jedrezejck, *et al.,* 2007).

First Generation Cephalosporins

They are very active against Gram-positive aerobic cocci. Example *Streptococci Pyogenes* (group A strep), *S. agalatiae* (group B) Viridians streptococci-except Enterococci, Methicillin resistant Staphylococci (MRSA) and *Streptococci Pneumonia.* But moderately active against some Gram-negative aerobic rods, primarily *Eschericia coli*, *Proteus mirabilis* e. t. c. and also anaerobic cocci (Jawetz, *et al.*, 2010).

Cephaloxin, cephradine and cefadroxil are absorbed from the gut to a variable extent and can be used to treat urinary and respiratory tract infections. Cefazolin is a drug of choice for surgical prophylaxis because it gives the highest (90 - 120μg/ml) levels with every 8 hour dosing whereas Cephalothin and Cephapirin in the same dose give lower levels (Jawetz, *et al*., 2010). It has a limitation of not effective against *Bacteroide faecalis*. None of the first generation drugs penetrate the central nervous system (CNS) (Jawetz, *et al,* 2010).

Second Generation Cephalosporins

Second generation agents are active against organisms covered by first generation drugs but have extended coverage against Gram-negative rods including *Klebsiella spp* and *Proteus spp* but not *Pseudomonas aeruginosa* (Jawetz, *et al.*, 2010).

Cefexitim and Ceforelan are active against *Bacteroides faecalis* and thus are used in mixed anaerobic infection including peritonitis or Pelvic Inflammatory Disease (PID). However, resistance to those agents among the *B. faecalis* group is increasingly (Jawetz, *et al*. 2010).

The Cephamycin group is useful for mixed aerobic/anaerobic infection of the skin and soft tissues, Intra abdominal, and gynaecology infections and surgical prophylaxis (Widmer, 2008). Only second generation drugs cross blood Gram barriers. The limitation of the second generation Cephalosporins is that they have more effect on community-acquired infections than hospital-acquired infections or complicated community-acquired infections. The Cephamycin agents have a side claim called the Methylthiotetrazole (MTT) group which predisposed patients to;

(i)   Hypoprothrombinemia and bleeding by disturbing synthesis of vitamin k dependent clothing factor

(ii) Alcohol intolerance by causing a disulfiram-like reaction, avoid alcohol products for several days after antibiotics have stopped.

(iii) Increase in resistance to *B. faecalis* group increases to these agents.

Third Generation Cephalosporins

The third generation Cephalosporins have a broad-spectrum of activity and further increased activity against Gram-negative organism such as the *Enterococci. Haemophilic influenza, Moraxella catarharlis Nesseria minigitidis Escherichia coli, Klebsiella specie* and *Proteus mirabilis.* etc found in hospital and community acquire infection and also active against *P. aeuruginosa* which is a frequent cause of hospital acquired pneumonia but have decreased activity against Gram-positive cocci except for *Streptococi pneumonias viridans strept* especially Penicillin resistant *Streptococcus pneumonia, Enterococci* which are intrinsically resistant to Cephalosporin and often produce super infections during their use (Pichichero, 2006). The limitation is that most third generation Cephalosporins such as Cefotaxime, Ceftriaxons, Ceftixone are active against Staphylococci but Ceftazidime is only weakly active (Jawetz, *et al*, 2010).

-   None of the agent is active against MRSA, *Enterococci* and *L.monocytogenes.*
-   Cephalosporins are not drug of choice for *Enterobacter* infection because some spp have a tendency to become resistant during cephalosporin therapy.

Fourth Generation Cephalosporins

Cefepime is the only fourth generation Cephalosporins now in clinical use in the United States. It has enhanced activity against *Enterobacter* and *Citrobacter* species that are resistant to third generation Cephalosporins.

Cefepime has the excellent activity against *Enterobacteriae* and *Pseudomonas aeruginosa* which are similar to ceftazidime in addition. It also has better Gram-positive activity than Ceftazidime.

BACTERIAL RESISTANCE TO CEPHALOSPORINS

During the past 15 years, emergence and dissemination of β-lactam resistance in nosocomial *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumanni*, became a serious problem worldwide. Especially the increasing resistance to third and fourth generation Cephalosporins and Carbapenems is of particular concern. Gram-negative bacteria pursue various molecular strategy the presence and properties for development of resistance to these antibiotics (Yvonne, *et al*., 2010).

Resistance of Gram negative bacteria to Cephalosporins, as with other beta-lactam antibiotics

is a function of a site (penicillin-binding proteins). Permeation through the outer- membrane is largely governed by the presence and properties of porins, which are water filled channels facilitating the movement of hydrophilic molecules across the membrane. The properties of porins vary considerably between wild-type bacteria species, and their members (and hence the ability of a bacterial cell to exclude antibiotic) may be reduce in strains with acquired resistance. In the case of cephalosporin, ability to cross the outer membrane is related to physiochemical properties such as molecular size, hydrophobicity and the number and charge of ionized group. Thus, for, example, dianionic compound have a general lower permeability rate than dipolar Cephalosporins. The phenotypically expressed susceptibility of a particular bacterial strains to cephalosporin is brought about by a dynamic combination of permeation, the ability of the agent to resist degradation of binding to the beta-lactamase in the periplasmic space which act upon the relatively low concentration of Cephalosporin present their and target affinity (chemother, 1996).

Resistance to Cephalosporins can develop as a result of any of the following processes:

(i) Microorganisms may lose the specific target structure for a drug for several generations and thus be resistant. For instance, Penicillin susceptible organisms may change to cell wall different L-forms during Penicillin administration. Lacking of cell wall, they are resistant to cell wall inhibitor drugs. Example Cephalosporins may remain so for several generations when these organisms revert to their bacteria parent forms by resuming cell wall production, they are again susceptible to Cephalosporin (Jawetz, *et al*., 2010).

(ii) Generation of extended –spectrum β-lactamase (ESBL) according to the original definition due to extension of the spectrum of already widely disseminated plasmid-encoded β-lactamase by amino acid substitution (Yvonne, *et al*., 2010).

(iii) Acquisition of gene encoding (ESBL) from environmental bacteria as for instance the CTX-M-Type β-lactamase from Kluyvera species (Yvonne, *et al*., 2010).

(iv) High level expression of chromosomal encoded β-lactamase (bla) genes, as blaoxa or blamp genes due to modification in regulatory genes mutations of the β-lactamase promoter sequence as well as integration of insertion sequences containing an efficient promoter for intrinsic bla genes (Yvonne, *et al*., 2010).

(v) Mobilization of bla genes by incorporation in integrous and horizontal transfer into other Gram-negative species such as the transfer of the ampc gene from *Enterobater freunchii* to *Klebsiella species* (Yvonne, *et al*., 2010).

(vi) Dissemination of plasmid-mediated carbenpenemases as KPC and metallo –β-lactamase (Yvonne, *et al*., 2010).

(vii) Non-expression of porin genes and/or efflux pump-based antibiotic resistant (Yvonne, *et al*., 2010).

Table1: Number/percentage of isolates from different sources.

Specimen (source)	number of organism isolated	Percentage of isolates
Urine	17	(28.3)
Blood	11	(18.3)
Stool	10	(16.6)
Wound swab	13	(21.6)
Ear swab	6	(10)
Throat swab	1	(1.6)
HVS	2	(3.3)
Total	6	100

METHODS OF PERFORMING SENSITIVITY TESTING TO ANTIBIOTICS

In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, sensitivity (susceptibility) testing is used to select effective antimicrobial drugs. In order to achieve this purpose, several methods are devised to determine the susceptibility testing of the isolated pathogen to the microbial agents. These methods includes:

- Dilution tests
- Diffusion tests

Dilution Tests Methods

This is a technique used in measuring the Minimum Inhibitory Concentration (MIC). It can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacterial (Cheesbrough, 2000).

To achieve the dilution technique, the following methods are used.

Broth (Tube) Dilution Method:

Here a medium which will support the growth of the test organism should be used. Mueller Hinton broth is most commonly used. Todd-Hewit broth may be used for organisms that do not grow well in Mueller-Hinton broth e.g. Streptococci (Ochei, *et al.,* 2007).

Table 2: Number/percentage of various bacterial isolates from   different sources.

Source	Klebsiella spp	S aureus	Escherichia coli	Proteus spp	Salmonella spp	Pseudomonas spp
Urine	6(40)	2(13.3)	6(60)	2(33.3)	0(0)	1(11.1)
Blood	3(20)	4(26.6)	1(10)	0(0)	0(0)	3(33.3)
Stool	(0)	0(0)	3(30)	2(33.3)	5(100)	0(0)
Wound swab	5(33.3)	4(26.6)	0(0)	2(33.3)	0(0)	2(22.2)
Ear swab	1(6.6)	2(13.3)	0(0)	0(0)	0(0)	3(33.3)
Throat swab	0(0)	1(6.6)	0(0)	0(0)	0(0)	0(0)
HVS	0(0)	2(13.3)	0(0)	0(0)	0(0)	0(0)
Total	15(25)	15(25)	10(16.6)	6(10)	5(8.5)	9(15)

Agar Dilution Tests

This method is similar to broth dilution tests except that varying concentrations of the antibiotics are incorporated in series of agar plates onto which a standardized suspension of the test organisms is inoculated. The drug dilutions should be 10 times the required concentration by mixing 2ml of each dilution with 18ml of molten agar at $50^{o}C$ per plates. After incubation, the lowest concentration of the agent which shows no growth of the test organism is MIC.

This procedure is cumbersome and it is not used routinely (Ochei, *et al,* 2007).

(i) Micro Dilution Test

This test is similar in principle to the tubes dilution test except that the test is performed in a series of wells in a microtitre plate.

Diffusion Test Method

Different methods are used for carrying out the diffusion test method.
- (i) Disc diffusion test
- (ii) Ditch plate method
- (iii) Healtley cup or punched hole diffusion method

Disc Diffusion Test

This is the most widely used antimicrobial susceptibility (sensitivity) tests in clinical laboratories. A disc of blotting paper impregnated with a known volume of appropriate concentration of an antimicrobial agent is placed on a sensitivity test agar inoculated with the test organism. The antibiotic diffuses into the surrounding medium establishing a gradient of concentration around the disc. The growth of the organism is inhibited up to a distance from the disc where the concentration of the drug is roughly equal to its minimum inhibitory concentration (MIC). The inhibition of growth appears as a circular zone of inhibition on the agar plate. The zone diameter is roughly proportional to the sensitivity of the test organism to the drug.

Two methods are commonly use in the disc diffusion method.
- (i) Kirby – Bauer's method
- (ii) Stokes's method

Kirby-Bauer's Method

In this method, the inhibition zone diameters of the test organisms are compared with those of the control strains with reference to the corresponding medium inhibition concentration and interpretive guidelines published by National Committee for Clinical Laboratories Standards (NCCLS).

Stoke's Method

This method is similar to that of Kirby Bauer except that the test organisms and control organism are tested against the same discs on the same plate. This eliminates many variables which may affect accuracy. This method helps to control the activity of each disc, and the inhibition zone of the organism to be compared directly with that of the control.

Ditch Method

This is the method which consists of removing a strip of agar from a plate and filling the gutter thus formed with agar containing the antibiotic require to be tested. The plate is allowed to set and several organisms can then be streaked at right angles to the ditch. This method is suitable when a large number of organisms are to be tested against one antibiotic. The big disadvantage of this method is that the plate must be prepared fresh every day. This method is no longer used in clinical laboratories.

Heatley Cup Or Punched Hole Diffusion Method

The use of this method for antibiotic susceptibility testing has become obsolete. The method consisted of filling several punched holes made in an agar plate; or the special cups (open at both ends) placed on the agar plate, which is already seeded with test organism. The holes or cups are filled with varying concentrations of the antibiotic being tested. The method is considered too laborious.

MATERIALS AND METHOD
STUDY AREA

The study was carried out at Medical Microbiology Laboratory Jos University Teaching Hospital (JUTH) Jos, Plateau State between November and April, 2011.

SAMPLE SIZE

A total of 60 isolates made up of 10 (16.6%) Escherichia coli, 15 (25%) Klebsiella Pneumoniae, 6 (10%) Proteus Spp, 15 (25%) Staphylococcus aureus, 5 (8.5%) Salmonella spp and 9(15%) Pseudomonas Spp isolated from different clinical specimens including Blood, Urine, swabs, and other body fluids were used for this study.

Table 3: General pattern of Resistance of different isolates to different generations of Cephalosporins. Number/percentage resistance to different generation of Cephalosporins and control

Organisms (isolates)	1st generation (Cephalexin)	2nd generation (Cefurozime)	3rd generation (Ceftriazone)	Control (Ceftezidime)
Klebsiella	5(33.3)	3(20)	1(6.6)	3(20)
S. aereus	2(13.3)	1(6.6)	0(0)	1(6.6)
E. coli	6(60)	2(20)	1(10)	2(20)
Proteus Spp	4(66.6)	3(50)	1(16.6)	2(33.3)
Salmonella spp	0(0) l	0(0) l	0(0) l	0(0) l
Pseudomonas spp	9(100) r	6(66.6)	1(11.1)	1(11.1)

Key:

r   =   Highest resistance = zone of inhibition < 10mm

l = Lowest resistance = zone of inhibition < 15mm (+++)

METHODOLOGY
Collection of Samples

Isolates were collected from different benches after overnight incubation and stored on Nutrient agar slants in a refrigerator at 4°C with regular subcultures to nutrient agar after every 3 weeks, (Okoli *et al*, 2006).

PROCESSING OF SAMPLES

After aerobic incubation at 37°C, the organisms stored on Agar slants were subcultured on MacConkey agar and incubated for 18-24 hours (overnight) to obtain discrete colonies.

IDENTIFICATION OF BACTERIAL GROWTH

The inoculated plates were examined the following day for growth. Cultures appearing pure were recorded and subjected to further biochemical tests to confirm their identities.

Discrete well separated colonies of both lactose and non lactose fermenters on MacConkey were inoculated into peptone water and incubated for 1 hour at 37°c for further identification test (biochemical tests).

The colonies that appear as mixed growth were also inoculated into peptone water and incubated at 37°C for 1 hour from which subcultures were made on MacConkey in order to obtain pure culture. From the purity plates, the suspected colonies were again inoculated into fresh peptone water and incubated for 1 hour at 37°C for further identification tests (Biochemical tests)

Gram Staining Techniques

Gram staining was done according to method as described in Cheesbrough (2006).

Motility Tests

Motility was done according to method as described in Cheesbrough (2006).

Biochemical Tests

They are carried out according to method as described by Cheesbrough, 2006

ANTIBIOTIC DISC PREPARATION
Procedure: -

Using an ordinary office two hole puncher paper discs with approximate diameter of 6.3mm where punched out one by one from the whatman number 1 filter paper. The disc where placed in glass universal bottles and sterilized at 160°C for one hour, allowed to cool and stored.

PREPARATION OF ANTIBIOTIC SOLUTION
Preparation Of Ceftriazone And Cefurozime

This antibiotic comes in 1gram and when diluted with 10mls of distilled water gave 10^6 (1000000µg)

1g = 1000mg = 1000000

Therefore when diluted per 10mls will give 1g = 10/1000000µg

Since 30000µg was prepared, each disc contain 30µg and 100 discs were prepared per 1ml in the assumption that 1 disc will absorb 0.01ml.

Therefore 30µg = 10/1000000 X 30,000 = 30/100 = 3:10

That is 3 part of the diluted antibiotic to 7 part of sterile diluent.

Preparation of Cephalexin

This antibiotic comes in 250mg/5mls, but 125mg was diluted with 10mls of distill water which gave a final dilution of 2 part of antibiotic to 7 part of distill water.

10 mls of the antibiotic solution was prepared and 1ml of the antibiotic solution was taken so that it contains 100 times the amount of antibiotics required in the disc. 1ml of the solution was added to

each bottle of 100 discs and as the whole of this volume will absorb, assume that each disc contains approximately 0.01ml. They were stored in the wet condition and retain their moisture (Robert, *et al.,* 1975)

Antibiotic Susceptibility Testing

Confirmed isolates (*Escherichia coli, Proteus spp, Klebsiella spp, Pseudomonas aeruginosa* and *Staphylococus aureus*) were subjected to antibiotic susceptibility by disc diffusion method using prepared antibiotics discs containing different 30μg of antibiotics, Cefalexin, Cefuroxine and Ceftriaxone.

Procedure:

- The isolates was picked using sterile wire loop and incubated into peptone water and incubated aerobically at 37°c for one hour.
- The peptone water culture was then flooded on dried nutrient agar plates and excess peptone water culture were drained into a discard jar.
- Prepared discs were placed and firmly pressed onto the plate using a sterile forceps. Control was equally placed.
- The plates were incubated aerobically at 37°c for 24 hours (Ochei, *et al,* 2007).

Zones inhibition less than or equal to 15mm and above were taken as absolutely sensitive (+++). Those zones of inhibition of 12-14mm and 10-12mm were taken as moderately sensitive (++) and (+) respectively. Those strains of isolates that have smaller zone of inhibition or grow up to the edge of the disc, were taken as being resistance (Cheesbrough, *et al*; 2004).

3.   Results And Disscussion

Sixty (60) bacterial isolates from Urine, Blood, Stool, Wound, Ear, Throat and High Vaginal swabs were analyzed in this study.

Table 1 shows the number/percentage of isolates from different sources 17(28.3%) from Urine, 11(18.3%) from Blood, 10(16.6%) from Stool, 13(21.6%) from Wound swab, 6(10%) from Ear swab 1(1.6%) from throat swab and 2(3.3%) from High Vaginal swabs.

Table 2 shows the different number of isolates from different sources and the isolates were *Klebsiella spp 15(25%), Staphylococcus aureus 15(25%), Escherichia coli 10(16.6%), Proteus spp 6(10%), Pseudomonas spp 9(15%)* and *Salmonella spp (5(8.3%).*

The number/percentage of isolates that were resistant different generation of Cephalosporins i.e. first (Cephalexin), second (Cefuroxime), third

generation,(Ceftriazone) and control (Ceftazidime) is shown in table 3. *Salmonella spp* show the lowest resistance to all the 3 generations of Cephalosporins with 0(%) while *Pseudomonas aeruginosa* shows the highest resistance to the 3 generation of Cephalosporins as follows 1st generation 9(100%), 2nd generation 6(66.6%) and 3rd generation 1(11.1%) and control 1(11.1%).

Resistance patterns of the urinary tract and systemic (tissue isolates) are shown in table 4 and 5. *Pseudomonas spp* showed the highest resistance to all the three generation of Cephalosporins. The Urinary tract isolates showed 1(100%) and systemic isolates showed 15(62.5%) while *Salmonella* showed the lowest resistance to all the three generations with 0(0%)

Table 4: Resistance/percentage pattern of Urinary Tract Isolates to the three generation of Cep halosporins. Resistance pattern/percentage

Urinary tract isolates	1st generation	2nd generation	3rd generation
Klebsiella spp	2(33.3)	1(16.6)	0(0)
Staphylococcus aureus	1(50)	0(0)	0(0)
E. coli	3(50)	2(33.3)	1(16.6)
Proteus spp	2(100)	1(50)	0(0)
Pseudomonas spp	1(100)	1(50)	1(100)

Cephalosorins is one of the most widely used antibiotics in the treatment of both gram positive and gram negative organisms.

In this study, out the total of 60 clinical isolates analyzed, 26(43.3%) showed resistance to 1st generation Cephalosporins, 5(8.3%) to second generation, 4(6.6%) to 3rd generation Cephalosporins while 9(15%) to the control which is also a second generation Cephalosporins.

The high number/percentage 9(100%) resistance of *pseudomonas spp* to all the generation of Cephalosporins is not surprising because *Pseudomonas spp* are known to exhibit inherent resistance to most antibiotics. However the resistance decreases as the generation progresses 1(11.1%). This confirms the effectiveness of the 3rd generation over the others and is in agreement with the work done by Pegler, et al 2007. that 3rd generation Cephalosporins are more active against both gram positive and gram negative organism.

Since most bacteria isolates analysed in this work are susceptible to 3rd generation Cephalosporins with a minimal rate of resistance, therefore it shows that the efficacy of 3rd generation (Ceftriazone) is still high, though 4th generation (Cefepime) could be more.

The resistance to 1st and 2nd generation Cephalosporins could be as a result of abuse of the drug because 1st and 2nd generation Cephalosporins comes in oral forms which can easily be taken while 3rd generation brand comes in injectable form which makes it difficult for one to inject him/herself. Also 1st and 2nd generations are cheaper than 3rd generation Cephalosporins. Despite the resistance of bacteria to 1st and 2nd generation Cephalosporins they could still be used if there is appropriate laboratory sensitivity testing done on the isolates since organisms such as Salmonella are still susceptible to the three generations. However, in the absence of that, 3rd generation Cephalosporins should be recommended. In order to avoid the crisis of drug resistance, the efficacy of antibiotic should be checked from time to time, by carrying out comparative studies as done in this study.

Table 5: Resistance and sources of systemic (tissue) isolates to the three generation of Cephalosporins. Isolates Resistance pattern/percentage to the different generations of Cephalosporins

	1st generation	2nd generation	3rd genertation
Klebsiella spp	2(22.2)	2(22.2)	0(0)
Staphylococcus aureus	7(53.8)	1(7.6)	0(0)
E. coli	2(50)	1(25)	0(0)
Proteus spp	2(50)	2(50)	1(25)
Pseudomonas spp	5(62.5)	3(37.5)	2(25)
Salmonella spp	5(0)	0(0)	0(0)

Corresponding Author:
Vivian Okemena Agbor
Department of Medical Microbiology
Federal School of Medical Laboratory Jos
Jos University Teaching Hospital
Nigeria P. M. B. 2253.
E-mail: lynnmaor09@gmail.com

REFERENCES
1. Alekshun, MN, and Levy SB (2007): Molecular Mechanisms of antibacterial multi-drug resistance *cell* 128(6) 1037-50. doi:10.10161j.cell.2007.03.004. *PMID* 17382878
2. Baker, E.J., and Silverton RE, London (2001): Introduction to Medical Laboratory Technology, 7th Edition. 318.
3. Bosch, E, and Rosich, L. (2008): The contributions of Paul Ehrlich to pharmacology: "a tribute on the occasion of the centenancy of his nobel prize" *Pharmacology* 82(3): 171-9 doi:10.1159/000149583 PMID 18679046.
4. British National Formulary, (2008): www.ncbi.nim.nih.gov/pmc/articles.
5. Calderon C.B, and Sabundayo B.P, (2007): Antimicrobial Classifications: Drugs for Bugs. In Schiwalbe. *Antimicrobial susceptibility Testing Protocols.* CRC Press, Taylor and Frances group. ISBN 978-0- 8247)-4100-6.
6. Cephalosporins and other beta-lactams (2008) *"British National Formulary* (56 ed.). London: *BMJ Publishing Group Limited* and *Royal Pharmacentical Society Publishing.* pp 295 ISBN 0-85369-778-7.
7. Cheesbrough, M (2004): District Laboratory practice in Tropical countries. Part 2 Cambridge University press pages 132-143.
8. Cowen, LE. (2008): "The evolution of fungal drug resistance; modulating the trajectory fro genotype to phenotype," Nat. Rev. microbial 6(3): 187-98. Doi: 10.1038/nrmicro 1835. PMD 18246082.
9. Davey, P.G. (2002): Antimicrobial chemotherapy". In ledingham JGG. Warrell DA concise oxford Textbook of medicine, Oxford University press p.1475. ISBN 978-0-19-262870-1. Dorlands medical Dictionary: antibiotic. Achieved from the original on 2010-11-17.
10. http.//www.webaitation.org/5ujytiomG.
11. Erdem, H, Kilic, S. *et al*, (2005); Gram negative bacterial resistance to cephalosporins in community acquired infection in Turkey. *Journal of antimicrobial chemother* 17(1): 61-65
12. http:/www.ncbi.nim.gov/pubmed/15828445.
13. FDA Rules override warnings about things, (2007).
14. Finberg, RW. Moellering, RC. Tally fp, etal; (2004): The importance of bactencidal drugs future directions in infectious diseases. Clinical Infectious Disease 39(9): 1314-20 doi-10.1086/425009 PMD 15494908.
15. Florey, H.W. (1945): use of micro-organisms for therapeutical purposes. B. med J.2 (4427). 635-642. doi: 10.1136/bmj.2 4427.635.PMID 20786386.
i. http://www.washingtonpost.com/wp-dyn/content/article/2007/AR2007030301311.html
16. James, P.A and Reeves, D.S, (1996): Bacterial resistance to cephalosporins as a function of outer membrane permeability and access to their target. *Journal of antimicrobial Chemother* 2:37-47 Jawetz, M. and Adelbergs, (2010): medical

microbiology 25th Edition, McGram Hill pg.339-361.

i. Jedrzeejczyk, Tadeusz, (2007): Internetowa Encyklopedia lekowlekimed. Pl. http://www.leki.med .pl/ek. phtm?id=428andidnlek = 2682&menu=4

17. Kenneth, T. (2008): online textbook of bactenology "The Good, the Bad and the Deadly". Science magazine, vol. 304:p1421. Department of Bactenolgy; University of Wisconsin, madiso.www. Textbook of bacteriology next.

18. Kingston, W (2008). Irish contributions to the origins of antibiotics. Irish journal of medical science 177 (2): 87-92.

19. Kollef, M.H. (2009): New antimicrobial agents for methicillin-resistant Staphylococcus aureus crit care Resusc 11(4): 282-6 PMID 20001879.

20. Kosinski, M.A, Joseph, W.S. (2007): update on the treatment of diabetic food infections. Clin podiatry med surg24(3): 383-96, vii doi 10.1016/j.Clin-PMID 17613382.

21. Limbird, L.E. (2004): The receptor concept: a continuing evolution Mol. Interview 4(6): 326-36 doi :10.1124/mi.4.6.6 PMID 15616162.

22. Lindblad, W.J. (2008): considerations for Determining if a Natural product is an Effective wound Healing Agent. *International Journal of lower Extremity wounds* 7(2) 75-81.

23. Marguez, B. (2005): Bacterial efflux systems and efflux pumps inhibitors. Biochimic 87:1137-1147.

24. Mattey, M., Spencer, J. (2008): *Bacteriophage therapy cookedgoose or phoemix rising curr. Opin. Biotechnol* 19(6): 608-12.

25. Ochei, J. and Kolhakfor, A (2007): medical laboratory science, Theory and practice, 5th Edition Fate McGram Hill production Ltd, New Delli, pg 788-789, 795-812.

26. Pearson, C. (2007): Antibiotic Resistance task Growing problem worldwide Voice of Africa http:/www.voanews.com.

27. Pegler, S. and Healy, B. (2007): In patients allergic to Penicillin, consider second and third generation Cephalosporins for lye threatening infections. *BMJ* 335 (7627): 991.

28. Pichichero, M. E. (2006): Cephalosporins can be prescribed safely for Penicillin–allergic patients (PDF*). The journal of family practice 55(2): 106-12*

29. Pirotta, M.V. and Garland, S.M. (2006): Genital Candida species detected in samples from women in Melbourne, Australia, before and after treatment with antibiotics *j.clin microbiology* 44(9). 3213-3217.

30. Podolsky, M. and Lawrence (1998): cures out of chaos Howunexpected Discoveries Led to Breakthroughs in Medicine and Health, Harwood Academic Publishers.

30. Richard, L.S, Ronald, S.G. (2009): Infectious Diseases of the female Genital Tract Lippincott Williams and Wilkins Pp 403. ISBN 9780781778152.

31. Rossi, S.Adelaide (2006): *Australian Medicines Handbook.*

31. Robert Cruickshank, J. P., Duguid, B. P., Marmion, R. H. A., and Swain (1975). Medical Microbiology: *The practice of Medical Microbiology,* 12th edition. Churchill Livingstone

32. Sheldon, V. (2005). Textbook of Bacteriology, McGraw Hill 589-509.

33. Slama, T.G, Amin, A., Brunton SA, et al. (2005): A clinician's guide to the appropriate and accurate use of antibiotics: the council for Appropriate and Rational Antibiotic Therapy (ARAT) criteria. AM.J Med. 118 7A. 15-65.

34. Stork, C.M. (2006): Antibiotics, Antifungals and Antivirals Goldtranks, toxicologic emergence. New York. McGraw Hill, Pg 847.

35. Sykes, R. (2001): Penicillin: From discovery to product. Bull world health organization 79(8): 778-9.

36. Von Nussbaum, F. *et al*, (2006): Medicinal Chemistry of Antibacterial Natural products-Exodus or Revival. *Angew chemi. Int. Ed* 45(31):5072-5129.

37. Waksman, S.A. (1947): What is an Antibiotic or an Antibiotic substance? Mycologia 39(5) 565-569. http://jstor.org/stable/3755196.

38. Widmer, A.F. (2008). Ceftobiprole: a new option for treatment of skin and soft-tissue infections due to methicillin resistant Staphylococcus aureus. Clin. Infect. Dis 46(5): 656-8.

39. Witte, W (2004). International dissemination of antibiotic resistance any practical conclusions? Curr.Opin. Microbiology 9(5): 461-5.

8/18/2011