Journal of Microbiology Research and Reviews
Vol. 4(3): 19-24, August, 2017
ISSN: 2350-1510

www.resjournals.org/JMR

Evaluation of the Resistance Pattern of *Escherichia Coli* and *Klebsiella Pneumoniae* Against Beta Lactam and Non Beta Lactam Antibiotics

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Abstract

This study was designed to determine *Escherichia coli* and *Klebsiella pneumoniae* resistance pattern to some beta and non-beta lactam antibiotics currently in use. Fifty (50) samples (25 stool sample and 25 sputum sample) were collected and cultured using streak method of inoculation. Fifteen (15) isolates of *Klebsiella pneumoniae* was gotten from sputum samples and 15 isolates of *E.coli* gotten from stool samples. Disc diffusion method was adopted for the antimicrobial assay. Out of the 15 isolates of *E.coli* tested, 93.3% were susceptible to ciprofloxacin, 80.0% to Gentamicin, 86.7% to Oflaxacin,73.3% to sulfamethoxazole and 80.0% to Nitrofurantoin, while all were resistant to amoxicillin. *Klebsiella pneumoniae* showed 80.0% susceptibility to ciprofloxacin, 73.3% to Gentamicin, 80.0% to Oflaxacin and 100.0% and 93.3% resistance to sulfamethoxazole and Nitrofurantoin respectively. For the beta-lactam antibiotics, 80.0% susceptibility to ceftazidime was recorded against *Klebsiella pneumoniae*. There was no significant difference (p=0.05) in activity between the beta-lactam drugs and the non-beta-lactam drugs against the isolates. Therefore, any of the drugs from these groups could be used in the treatment of infections caused by these test isolates.

Keywords: Resistance pattern, beta lactam antibiotics and non-beta lactam antibiotics, Evaluation.

INTRODUCTION

Many of the 2nd and 3rd generation cephalosporins and penicillines were specifically designed to resist the hydrolyte action of major β-lactamases. However, new β-lactamases emerged against each of the new classes of β-lactam that were introduced and caused resistance. Infact, since β-lactam antibiotics come into clinical use, β-lactamases have co-evolved with them, (Tangden, 2010). Among the wide array of antibiotics, β-lactam are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use (Brownson and Barret, 2001). But despite worldwide use of β-lactam antibiotics, the distribution of the enzymes responsible for resistance to oxyimino-cephalosporins and carbapenemes is far from being uniform ESBLS have spread widely and constitute a major cause of nosocomial infections associated with high mortality rates,particularly in serious infections such as septicemia (Kim *et al.,* 2010). ESBL producing strains have variable susceptibility rates for fluoroquinolones, amonoglycosides and fourth generation cephalosporins (Paterson, 2006).

In Africa, extended spectrum beta lactamases have been reported in Egypt (Shannon and French, 2004). Tunisia, Senegal (Broson, 2001). Nigeria (Soge *et al.,* 2006). South Africa (Essack*et al.,* 2001). Kenya (Kariuki*et al.,* 2001) etc. from different clinical sources.Emerging resistance to antimicrobial drugs increases morbidity and mortality by hampering the provision of effective chemotherapy and makes treatment more costly (Shannon and French , 2004).

These ESBL producing organisms vary in their susceptibility to different beta and non-beta lactam antibiotics and despite resistance to some they may appear sensitive to others. (George *et al.,* 2005). *E.coli* and related bacteria constitute about 0.1% of gut flora, fecal oral transmission is the major route through which pathogens strains of the bacterium cause diseases. Cells are able to survive outside the body for a limited amount of time which makes them ideal indicator organisms to test environmental samples for fecal contamination (Ishii *et al.,* 2008). As a member of the enterobacteriaceae family, *E. coli* does not usually occur naturally on plants or in soil and water. Within human and animal faeces, *E. coli* is present at very high concentrations (George *et al.,* 2005). And comprises about 1% of the total biomass in the large intestine.Although *E. coli* are part of the natural faecal flora, some strains of this bacterium can cause gastrointestinal illness along with other more serious health problems. *E. coli* like other number of Enterobacteriaceae family, produces or manufactures an enzyme called extended spectrum beta lactamases which are antibiotics resistant. (Spamu*et al.,* 2002). The ESBL produced by some *E.coli* strains are resistant to beta lactam antibiotics and other classes of antibiotic such as macrolides quinolones and Aminoglycosides. (Paterson*,* 2006*)*. ESBL are also plasmid- mediated enzymes and they carry resistance genes from one antibiotic to another, thus Gram negative bacilli containing these plasmids are multi-drug resistant. Furthermore, these plasmids are mobile genetic elements and can be transmitted between gram negative bacilli of different species in vivo (Paterson, 2006).

Members of the *Klebsiella genus* typical express 2 types of antigen, O-antigen is a component of the lipopolysaccharide (LPS) of which a varieties exist. The second is K-antigen, a capsular polysaccharide with more than 80 varieties (Tangden, 2011). Both contribute to pathogenicity and form the basis for sero-grouping.*Klebsiella pneumoniae* is second only on *E.coli* as a urinary tract pathogen, *Klebsiella* infection are encountered far more often now than in the past. This is probably due to the bacterium's resistance properties.Some *Klebsiella* bacteria have become highly resistant to antibiotics when bacteria such as *klebsiella pneumonia* produce an enzyme known as a carbapenemase (referred to as KPC producing organisms), then the class of antibiotics called carbapenems will not work to kill the bacteria and treat the infection. Unfortunately carbapenem antibiotics often are the last line of defense against Gram negative infections that are resistant to other antibiotics (Paterson*,* 2006). Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β- lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid.

The aim and objectives of this work was to determine the effectiveness of beta-lactam and non-beta-lactam antibiotics against Gram negative bacteria notably *Escherichia coli* and *Klebsiella pneumonia*, and finally to compare the effectiveness of the two group of antibiotics.

MATERIALS AND METHOD

Sample Collection

Stool and sputum samples were collected with sterile disposable container from students of Michael Okpara University of Agriculture, Umudike. A total number of 50 clinical specimens (25 stool and 25 sputum samples) werecollected.

Bacterial Isolation

The samples were incubated on the agar plate (Eosine Methylene Blue, Nutrient, MacConkey agar) with the aid of a sterile wire loop by streak plate method. It was incubated at 37^{o}C for 24 hours. The growth of microorganisms in the culture plate were examined for the various morphological characteristics which include shape, size, elevation, surface, edges, colony structure, degree of growth and nature.

Identification and Characterization of Bacterial Isolation

Bacterial isolates were characterized and identified using two step approach namely biochemical test and Gram staining. The characteristic features of the colonies observed were noted. The biochemical tests carried out were indole and citrate utilization tests.

Antibiotics Susceptibility Test

Muller Hinton agar was prepared according to the manufacturers instruction, after a colony of the test organism were collected and swab homogenously on the surface of an already prepared media- which has been tested for microbial contamination after 24 hrs of preparation on them. Different antibiotics dics were used which include the beta-lactam

Table 1. Percentage occurrence of isolates in stool and sputum samples

Clinical Specimen	No of sample collected	*Klebsiella pneumoniae* isolates	*E.coli* isolates
Stool	25	0 (0%)	15 (60%)
Sputum	25	15 (60%)	0 (0%)
Total	**50**	**15 (60%)**	**15 (60%)**

Table 2. The Resistant Pattern of the Test Organisms to the Drugs

Antibiotics	*Escherichia coli*			*Klebsiella pneumoniae*		
	Resistant	Intermediate	Sensitivity	Resistance	Intermediate	Sensitivity
Non-Beta-lactams						
Gentamicin	1(6.7%)	2(13.3%)	12 (80%)	4(26.7%)	0(%)	11(73.3%)
Ofloxacin	0(0%)	2(13.3%)	13(86.7%)	1 (6.7%)	2 (13.3%)	12(80%)
Sulfamethoxazole	1(6.67%)	3(20%)	11(73.3%)	9(60%)	6(40%)	0 (0%)
Nitrofurantoin	3(20%)	0(0%)	12(80%)	13 (86.7%)	1(6.7%)	1(6.7%)
Beta-lactams						
Amoxicillin	12 (80%)	3(20%)	0(0%)	11(73.3%)	0 (0%)	4(26.7%)
Ampicilin	12(80%)	1(6.7%)	2(13.3%)	11(73.3%)	0 (0%)	4(26.7%)
Ciprofloxacin	1(6.7%)	0(0%)	14(93.3%)	3(20%)	0 (0%)	12(80%)
Ceftazidime	6(40%)	0(0%)	9(60%)	2(13.7%)	1(6.7%)	12(80%)
Cefotaxime	3(20%)	2(13.3%)	10(66.7%)	11(73.3%)	2 (13.3%)	2(13.3%)

and non-beta lactam antibiotics. The beta-lactam antibiotics include, Amoxicillin, Ampicillin, Ceftazidime, Cefotaxime and Ceftriaxone and the non-beta-lactam include Gentamicin, ofloxacin, nitrofurantoin etc. Commercially prepared antibiotic (beta-lactams and non-beta-lactams) discs were used.. A sterile forcep was used to place each of the antibiotics on the surface of the prepared media at a distance of 5mm and then incubated for 24 hrs at 37^{o}C. After 24hrs of incubation, zones of inhibition were measured in and recoreded in mm using a meter rule. Zones of about 20mm and above were regarded as highly sensitive, 17-19mm as sensitive, 14-16mm as moderately sensitive, and 13mm or less as resistant (CSLI, 2015).

METHOD OF DATA ANALYSIS

Data collected were subjected to statistical analysis. One way Analysis of Variance (Anova) was used to test and compare the activities of the both groups of antibiotics against the different bacterial isolates.

RESULTS

Out of 50 specimen collected (stool, 25 specimens and sputum, 25 specimens), a total of thirty (30) isolates [*Escherichia coli* (15) and *Klebsiella pneumoniae*(15)] were isolated and identified as shown in table 1.
Invitro antibiotic susceptibility activities of ten different antibiotics from beta lactam and non-beta lactam groups against the bacterial isolates are presented in Table 2. *E.coli* showed 80.0% resistance to Amoxicillin and Ampicillin , it further showed 40.0% resistance to Ceftazidime and Ceftriaxone and was sensitive togentamicin (80.0%), Ciprofloxacin (93.3%), Ofloxacin (86.7%), Nitrofurantoin (80.0%), and Sulfamethoxazole (73.3%). *Klebsiella pneumoniae* showed 73.3% resistance to Amoxicillin, cefotaxime ceftriaxone and Ampicillin, then 86.7 and 60.0% resistance to Nitrofurantoin

Table 3. Comparison of the Effect of the Drugs on *E. coli* and *Klebsiella pneumoniae*

Source	SS	DF	MS	F	S
Between groups	43	1	45	2.26	4.41
Within error	359	18	19.9		
Total	404	19			

KEY: F = Frequency, SS= Sum of squares, DF= Degree of freedom, MS=mean square, S= Significance

Table 4. Comparison of the Activities of the Two Groups of Drugs (Beta-Lactam and non-Beta Lactam)

Source	Sum of Squares	Degree of Freedom	Mean Square	F	Significant
Between groups	162.55	3	54.18	2.83	4.41
Within error	306	16	19.12		
Total	468.55				

KEY: F = Frequency

Table 5. Antibiotic sensitivity comparism between *E.coli*and *Klebsiella pneumoniae*

Test organism	Antibiotics									
	AMC	CN	OFL	SXT	F	AMP	CIP	CA2	CFO	CFT
E.coli	_	+++	+++	+++	+++	-	+++	++	++	++
Klebsiella pneumoniae	_	_	_	_	_	_	+++	+++	-	-

KEY:
AMC – Amoxicillin , +++ = Highly sensitive , CN – Gentamicin , ++ = Moderately sensitive, OFL – Ofloxacin , + = Sensitive, SXT – Sulfamethoxazole/trimethoprim, - = Resistance, F – Nitrofurantoin, AMP – Ampicillin, CIP – Ciprofloxacin , CAZ – Ceflaxidime, CFO – Cefotaxime

and sulfamethoxazolerespectively. *K. Pneumoniae* showed 80.0% sensitivity to ofloxacin , ciprofloxacin , ceftazidime and 73.3% sensitivity to Gentamicin .

The antibiotic resistance patterns of *Klebsiella pneumoniae* and *E. coli* were compared as shown in table 5.It was observed that the activities of the drug on *Klebsiella pneumoniae* and *E.coli* were not significantly different at p=0.05. This was confirmed by one way anova statistical analysis adopted in Table 3. At p value 0.05 the difference between the resistance of the two isolates were found to be insignificant, indicating that the drugs have an average equal effect on the two isolates.

A one way Anova was employed again to compare the activities of the Beta-lactam and non-beta lactam antibiotics on the isolatesat p value of 0.05 confidence level. It was observed that there was no significant difference on the efficacy of the two group of drugs as shown in Table 4

DISCUSSION

Extended spectrum beta-lactamase producing organisms vary in their susceptibility to different antibioticsand despite resistant to some they may appear sensitive to others (George *et al*., 2005). The study has shown that there may be the possibility of production of new Beta-lactamases by bacterial isolates. This was demonstrated by very poor bacterial susceptibility to amoxicillin by all the isolates. This correspond to the observation made by Spamu *et al*., (2002), which indicated that *Klebsiella pneumoniae* and *E. coli* were resistant to penicillin and can acquire resistance to the third and fourth generation owning to the production of plasmid-mediated extended spectrum beta–lactamase. In the study

conducted by Spamu *et al*., (2002), gentamicin and tribramycin typically demonstrated poor *in vitro* activity against ESBL-producing organisms. However in this present study gentamicin, the only aminoglycoside used in this study showed appreciable *in vitro* activities against *E.coli* as well as improved *in-vitro* activities against *Klebsiella pneumoniae*. This is in line with observation made by Spamu *et al*., (2002). However, the resistance of these isolates to some of the antibioticsis subject to serious concerns since the therapeutic options are limited.

The significant low resistant of the isolate to gentamicin is important because gentamicin is traditionally considered as the first line drug against Gram- negative bacilli on the hospital setting. Penicillins are bactericidal; they inhibit bacterial cell synthesis. (Trevor and Katzung, 2001). *E.coli* and *Klebsiella* showed low susceptibility to Ampicillin which is in line with the study of Farhat *et al*., (2009). The observed resistance of *Klebsiella pneumoniae* and *E. coli* to ciprofloxacin was 6.7% and 20% respectively with sensitivity of 93.3% and 80% respectively. This was however not in agreement with the findings of Revathi *et al* (1998).

The observed resistance of *Klebsiella pneumoniae* on sulfamethoxazole is creating a vicious cycle, increasing the development of resistance which was in line with already established fact that *Klebsiella pneumoniae* is resistant to sulfamethoxazole (Spamu *et al*., 2002). The high susceptibility observed when *E. coli* is treated with nitrofurantoin is in line with observation made by Xu *et al*., (2016) indicating that Nitrofurantoin is good for treating diseases resulting from *E. coli* infection especially in urinary tract infections.

Ceftazidine showed a high activity against both *E. coli* (60%) and *Klebsiella* (80%). This is in line with the findings of Okesola *et al*., (2009). Ceftazidine also showed an inhibitory effect of 80% on *Klebsiella pneumoniae. E. coli* showed high sensitivity to Cefotaxime, (66.7%) a third generation of cephalosporin. Ceftriaxone also showed inhibitory activity agaist *E. coli* but however, was highly resisted by *Klebsiella pneumoniae.*

There was no significant difference between the resistance pattern showed by *Escherichia coli* and *Klebsiella pneumoniae* according to the one wayAnova statistical analysis employed at 95% confidence interval. This can be attributed to the fact that while some drugs have strong *in-vitro* activities on *Klebsiella pneumoniae* the other has strong activities on *E. coli.* To buttress this fact, another statistical analysis was conducted using one way Anova but this time the comparison was between the beta-lactam groups and non-beta-lactam groups. At p value of 0.05, no significant difference was observed on the susceptibility of the isolate to beta-lactam and Non-beta-lactam groups, indicating that both groups of drugs have similar inhibitory activity on the isolates (Ishii *et al.,* 2008 and Tangden, 2012).

However, this is a collective generation because certain drugs like Gentamicin, ciprofloxacin and oflaxacin have an outstanding effect on both *Klebsiella pneumoniae* and *E. coli* when compared with the other non-beta lactam drugs like the sulfamenthoxazole and Nitroflurantion which is only sensitive to *E. coli*. ceftazidime beta-lactam has a more sensitive effect on both *E. coli* and *Klebsiella pneumoniae* when compared with non-beta lactam drugs.

This study confirms the observation of Spamu *et al.,* (2002), that showed that resistance exhibited by *Klebsiella pneumoniae* and *E. coli* may be attributed to excessive and uncontrolled usage of antibiotics against the bacteria. This resistance by isolates posses' serious problems to treatment of infections caused by these bacteria since there are limited therapeutic options, thus, controlled usage of the antibiotics should be recommended.

CONCLUSION

This increased resistance by isolates indicates that amoxicillin and ampicillin are less effective in the treatment of *E. coli* and *K. pneumoniae* implicated infections and as such, other beta and non-beta-lactam drugs should not be administered without proper and comprehensive antibiotics susceptibility testings.

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