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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI FROM DIARRHOEA STOOL OF SOME CHILDREN ATTENDING GENERAL HOSPITAL DUTSE JIGAWA STATE.

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Abstract

Antibiotic susceptibility pattern of Escherichia coli isolated from diarrhoea stool samples of some children of age between 1-5 years attending General Hospital Dutse Jigawa State was carried out. Thirty two (32) out of Fifty (50) diarrhoea stool samples collected between March and April, 2016 were confirmed to be E. coli(64%). Twenty (20/32) of the samples were tested against selected antibiotics using Kirby-Baur disc diffusion technique to determine the susceptibility pattern of each. They were most susceptible to ofloxacin and least susceptible to nalidixic acid. Most of the isolates were found to be multi-antibiotic resistant. The MIC results showed that the minimum concentration at20mg/ml,10mg/ml, and 5mg/ml of ofloxacin inhibited the growth of one, three, and sixteen E. coli isolates respectively, where as the MBC results showed the minimum concentration of ofloxacin that indicated no growth of five, ten, and five E. coli isolates respectively. High rate of E. coli isolation from this study suggested that it might be the main cause of diarrhoea among children in Dutse. Highest potency of ofloxacin among the antibiotics tested suggests its most effectiveness in the treatment of diarrhoea caused by E. coli among this group of patients attending the hospital. Appropriate measures

should be taken by parents and care-givers to prevent contamination of food and water by enteric pathogen to avoid frequent occurrences of diarrhoea.

Key words: *Antibiotics, Diarrhoea, Hospital, and Susceptibility pattern*

INTRODUCTION

Diarrhoea is the passage of loose or watery stools, usually at least three times within a period of 24 hours (Sinclair *et al*., 2003). It is known to be one of the leading causes of illnesses in young children in developing countries (Parashar *et al*., 2003). Nearly half of deaths from diarrhoea among young children occur in Africa where diarrhoea is the largest cause of death among children under 5 years old and a major cause of childhood illness (WHO, 2007; Bezatu *et al*., 2013). The public health significance of diarrhoeal diseases cannot be overemphasized. Reports have shown that in Nigeria, more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease (Parashar *et al*., 2003). Diarrhoea is most prevalent in poor populations and in immune compromised individuals and the main etiology of the diarrhoea disease is related to a wide range of bacteria most of which are enteric parasites and viruses (Afroza *et al*., 2013). They include *Escherichia coli*, *Shigella, Salmonella* and *Campylobacter* (Suan *et al*., 1985), and the viruses: cytomegalovirus, Norwalk, hepatitis, herpes simplex virus, and rotavirus which seems to be the most common (Afroza *et al*., 2013).In developing countries, it is often associated with diarrhoeal infection during the time of weaning (Afroza *et al*., 2013).

Other sources of diarrhoea might include food intolerance, reaction to medicines such as antibiotics and antacids containing magnesium, ingestion of cosmetic products (Mwambete and Joseph, 2010).

The main cause of death from acute diarrhoea is dehydration, which results from loss of fluid and electrolyte in stool (Akingbade *et al*., 2013). There is the current problem of emergence of strains of *E. coli* implicated in food contamination that are resistant to most of the present antibiotics such as nalidixic acid, ampicillin. (Akingbade *et al*., 2013).
Antibiotic resistance has emerged and is attributed to abuse and misuse of antibiotics (Prescott *et al*., 2008; Manicanda and Amsath, 2014).Antibiotic drug resistance is an increasing threat to global health security, potentially compromising gains made in public health worldwide (Howell, 2013).

The main mechanisms of drug resistance include drug in activation or modification, alteration of target site, alteration of metabolic pathway and reduced drug accumulation by decreasing drug permeability and/or increasing active efflux of the drugs across the cell surface (Ash *et al*., 2002).

Antibiotics are among the most frequently prescribed medications in modern Medicine in which some may be either bactericidal or bacteriostatic (Yury, 2011). Antibiotics can be used

to treat a wide range of infections and are known as 'broad-spectrum' antibiotics or only effective against a few types of bacteria and are called "narrow-spectrum" antibiotics (Yury, 2011). It has been known for quite a long time that antibiotics were effective in the treatment of bacterial infections and susceptibility of different microorganisms to a given antimicrobial is variable and bacteria sometime become resistant to which they were originally sensitive. It is therefore a common practice in human medicine to determine whether the drugs to be in combating infection may be expected to inhibit or kill the causative organisms (Arzai, 2008).

The current antibiotic resistant of microbial species, for example, *Pseudomonas* and *Klebsiella species* resistance to antibiotics as well as toxicity to conventional therapy among other factors necessitate this study. The services of microbiologist and microbiological laboratory are often required to identify the pathogenic microorganisms and to give a guide to the clinical management and public, for example on the range of antibiotics that might be clinically effective. Most pathogens are known to be sensitive to some antibiotics while others are not, for example *Streptococcus pyogene* is always sensitive to Benzyl penicillin and *Escherichiacoli* is regularly resistance. However, sensitivity to any antibiotic may vary in different strains of the same species and might alter during the cause of infections, hence sensitivity test is often needed, (Karayil *et al*., 1998).

Patients who are infected with resistant bacteria, that are often resistant to multiple antibiotics (multi-drug resistance), have limited options for treatment, leads to increasing healthcare costs, extra length of stay in the hospital, treatment failures, and sometimes death. (Stockholm, 2013). As a result, this study was conducted to determine the antibiotic susceptibility pattern of *E. coli* from diarrhoea stool of children attending General Hospital Dutse

MATERIALS AND METHOD

Area of Study

The study was conducted in Dutse General Hospital. Dutse is the capital of Jigawa state, located between latitude 11˚ 42^{1} 04^{11} north and longitude 9˚ 20^{1} 31^{11} east of the equator.
Twenty*Escherichia coli*isolated from diarrhoea stool samples from children of age 1-5 years were subjected to antibiotic susceptibility test and pattern. Suspension of each bacteria was made using peptone water and compared with Mc-Farland's turbidity standard scale number 0.5. The bacterial suspension was continuously diluted using normal saline until the turbidity conforms to that of the Mc-Farland's scaleused as standard inoculum.

Susceptibility to antibiotics was carried out using Kirby Bauer disk method. About 0.1ml of standard inoculum was measured and seeded on a solidified nutrient agar plate using sterile swap stick. A Multiple antibiotic disc containing ten different antibiotics: Ofloxacin(10ug), Perfaxin(10ug),Ciproflox(10ug), Augmentin(30ug), Gentamycin(10ug), Streptomycin(30ug), Ceporex(10ug), Nalidixic acid(30ug), Septrin(30ug) and Ampicilin(5ug) was placed gently at the center of the plate by using sterile forceps. This was repeated for the 19 other isolates. The plates were incubated at 37^{0}C for at least 18 hours after which they were examined for zone

of inhibition. Any antibiotic that inhibited bacterial growth has a clear zone of inhibition and considered as being susceptible to, while those without any clear zone of inhibition were considered being resistant to by the organism. The results were expressed according to the criteria developed by Clinical and Laboratory Standard Institute (CLSI, 2013) and manual of antimicrobial susceptibility testing guideline (Cayle, 2005; Cheesbrough, 2010).

Minimum Inhibitory Concentration of the antibiotic was carried out using broth dilution method. Sterilized nutrient broth (10ml) was dispensed into a labeled test tube and 5ml was dispensed into each of the three other test tubes.Two-fold serial dilutions of an antibiotic in the broth were made to obtain the concentrations of 20.0mg/ml, 10.0mg/ml, 5.0mg/ml and 2.5mg/ml respectively. The highest concentration was obtained by dissolving 0.2g of an antibiotic in 10ml of the nutrient broth. Having obtained the different concentrations of an antibiotic in the broth, 0.1ml of the standard inoculum of the test bacteria in the normal saline were inoculated into the different dilution of an antibiotic in the broth, test tubes of the broth were incubated at 37^0C for 24 hours, the lowest concentration of an antibiotic in the broth which inhibited the growth of the bacteria was recorded as the Minimum Inhibitory Concentration (MIC). The results were expressed according to the guideline (Cheesbrough, 2010).

The Minimum Bactericidal Concentration of the Antibiotic (MBC)was carried out to determine whether the tested bacteria were killed or inhibited. The tubes of the MIC in the serial dilution that show no growth were sub cultured ontoprepared solidified nutrient agar and the plates were incubated at 37^0C for 24 hours after which the plates were observed for bacterial growth. The MBC was the plate with lowest concentration without colony growth. The results were expressed according to the guideline (Cheesbrough, 2010).

RESULTS

The result of Antibiotic susceptibility test on *E. coli* isolated from diarrhoea stool of children during the period of study is shown in Table 1. The number and percentage of*E. coli* isolates susceptible, intermediate and resistant to the selected antibiotics were indicated. The result showed that *E. coli* isolates 18(90%) were most susceptible to ofloxacin among other antibiotics tested, followed by reflacin 15(75%) and streptomycin 15(75%). The bacteria were least (80%)susceptible to nalidixic acid followed by ceporex 12(60%) and ampicilin 10(50%) respectively.

The results of antibiotic susceptibility pattern of*E. coli* isolated is shown in Table 2 which indicated the antibiotic combination, resistance pattern, number of *E.coli* resistance to a particular antibioticand multiple antibiotic resistance index (MARI) respectively. More so multiple antibiotic resistance index (MARI) was calculated using a formula: number of antibiotic that are resistance to a particular isolate divided by the total number of antibiotics used. It was observed that ten of the *E. coli* isolates were resistant to two different set of combinations of antibiotic which included: AUG, AMP; AUG, NAL; CEP, AMP; CEP, NAL;

CPX, STR; NAL, AMP. Based on this result, using MAR index, they are considered to be multiple antibiotic resistance. Ten of *E. coli* isolates were resistant to one set of three

Table 1: Antibiotic susceptibility testing on *E. coli* isolates from diarrhoea stool of some children.

Antibiotic	Susceptible (%)	Intermediate (%)	Resistance (%)
		N=20	
Ofloxacin (OFX)	18(90)	1(5)	1(5)
Replacin (REP)	15(75)	I(5)	4(20)
Ciprofloxacin(CPX)	1(5)	16(80)	3(15)
Augmentin(AUG)	6(30)	6(30)	8(40)
Gentamycin (GEN)	10(50)	8(40)	2(10)
Streptomycin(STR)	15(75)	4(20)	1(5)
Ceporex (CEP)	1(5)	7(35)	12(60)
Nalidixic acid (NAL)	0(0)	4(20)	16(80)
Septrin (SXT)	2(10)	18(90)	0(0)
Ampicilin (AMP)	3(15)	7(35)	10(50)

N: Total number of *E. coli* tested for antibiotic susceptibility test

antibiotic combinations (PEF, AUG, NAL) with MAR index of 0.3 indicating that the *E. coli* is multiple antibiotic resistant. Six of the *E. coli* isolates were found to be resistant to five sets of 4 combination of antibiotics including: AUG, CEP, NAL, AMP; CPX, CEP, NAL, AMP; AUG, GEN, CEP, NAL; CPX, CEP, NAL, AMP; PEF, AUG, CEP, NAL with MAR index of 0.4 indicating that they are multiple antibiotic resistant. One of the *E.coli* isolates was resistant to one set of five antibiotic combinations (PEF, AUG, CEP, NAL, AMP) with MAR index of 0.5 indicating that the *E. coli* is multiple antibiotic resistant. It is noteworthy that one of the *E. coli* isolates was resistant to a set of 8 combinations of antibiotics: OFX, PEF, CEP, AUG, GEN, CEP, NAL, AMP with MAR index of 0.8. This bacterium is considered to have a very high level of resistance to multiple antibiotics and considered as the most resistant.

Table 2: Antibiotic susceptibility pattern of the *E. coli* isolated from diarrhoea stool of children.

Antibiotic Resistance Pattern	**No. of *E. coli***	**MARI**
AUG, AMP.	1	0.2
AUG, NAL.	1	□□
CEP, AMP.	2	□□
CEP, NAL.	3	□□
CPX, STR.	1	□□
NAL, AMP.	2	□□
PEF, AUG, NAL.	1	0.3
AUG, CEP, NAL, AMP.	2	0.4
CPX, CEP, NAL, AMP.	1	□□
AUG, GEN, CEP, NAL.	1	□□
CPX, CEP, NAL, AMP.	1	□□
PEF, AUG, CEP, NAL.	1	□□
PEF, AUG, CEP, NAL, AMP.	1	0.5

OFX, PEF, CEP, AUG, GEN, CEP, NAL, AMP.	1	0.8

OFX : Ofloxicin, REP : Replacin, CPX : Ciproflaxacin, AUG : Augmentin, GEN : Gentamycin, STR : Streptomycin , CEP : Ceporex, NAL : Nalidixic acid, SXT : Septrin and AMP : Ampicilin.

The results of minimum inhibitory concentration test of ofloxacin at different concentration on*E. coli* isolated from diarrhoea stool of children during the period of study was as shown in Table 3. It was observed that 20.0mg/ml concentration of antibiotic (Ofloxacin) was the lowest that showed no turbidity (no growth) in *E. coli* isolate number 5 and therefore, 20mg/ml was found to be the MIC of the above mentioned isolate. 10mg/ml was the least concentration of antibiotic that tend to inhibit the growth of *E. coli* isolates number 4, 11 and 18 respectively and therefore 10.0mg/ml was the MIC of number 4, 11 and 18 isolates. 5mg/ml was the least concentration of antibiotic (ofloxacin) that inhibit the growth of *E. coli* isolates number 1-3, 6-10, 12-17 and 19-20 and therefore, 5mg/ml was found to be the MIC of the above mention isolates. The frequent occurrence of MIC was found in the concentration of antibiotic 5.0 mg/ml in the total of twenty *E.coli* isolates follow by 10.0mg/ml and then 20mg/ml respectively.

The results minimum bactericidal concentration of ofloxacin at different concentration on different *E. coli* isolates from diarrhoea stool of children during the period of study was shown in Table 4. It was observed that from the results obtained 20.0mg/ml was the least concentration of antibiotic (ofloxacin) that shows no growth on the plate of *E. coli* isolates number 3, 5, 9-10 and 15 and therefore, 20mg/ml was found to be the MBC of the above mentioned isolates. 10mg/ml was the least concentration of antibiotic that shows no growth on the plate of *E. coli* isolates number 4, 6, 8, 13-14 and 16-20 respectably and therefore, 10.0mg/ml was the MBC of the above mentioned isolates. The least concentration (5mg/ml) of antibiotic (ofloxacin) that shows no growth on the plate of *E. coli* isolates number 1-2, 7 and 11-12 were observed respectively and therefore, 5mg/ml was found to be the MBC of the isolates.

Table 3: Minimum Inhibitory Concentration of the Antibiotic (MIC)

E. coli	Concentration of antibiotic (mg/ml)				
	20	10	5	2.5	Negative control
1	-	-	MIC	++	++
2	-	-	MIC	++	++
3	-	-	MIC	++	++
4	-	MIC	+	++	++
5	MIC	+	+	++	++
6	-	-	MIC	++	++
7	-	-	MIC	++	++
8	-	-	MIC	++	++
9	-	-	MIC	++	++
10	-	-	MIC	++	++
11	-	-	MIC	++	++

12	-	-	MIC	++	++
13	-	-	MIC	++	++
14	-	-	MIC	++	++
15	-	-	MIC	++	++
16	-	-	MIC	++	++
17	-	-	MIC	++	++
18	-	MIC	+	++	++
19	-	-	MIC	++	++
20	-	-	MIC	++	++

MIC:Minimum Inhibitory Concentration, -: No turbidity, +: Turbid, ++: Highly turbid.

Table 4: Minimum Bactericidal Concentration of an Antibiotic(MBC)

E. coli	Concentration of antibiotic (mg/ml)		
	20	**10 5**	
1	_	_	MBC
2	_	_	MBC
3	MBC	+	++
4	_	MBC	+
5	MBC	+	++
6	_	MBC	+
7	_	_	MBC
8	_	MBC	+
9	MBC	+	++
10	MBC	+	++
11	_	_	MBC
12	_	_	MBC
13	_	MBC	+
14	_	MBC	+
15	MBC	+	++
16	_	MBC	+
17	_	MBC	+
18	_	MBC	+
19	_	MBC	+
20	_	MBC	+

MBC: Minimum bactericidal concentration, - : No growth from MIC, +: growth and ++: Highly growth.

DISCUSSION

The high susceptibility rate of *E.coli* against the antibiotics: ofloxacin 18(90%), reflacin15 (75%) and streptomycin 15(70%) might probably be as a result of infrequent use of those antibiotics to treat diarrhoea patients in the Hospital. This might be due to high cost of the drugs and certain restrictions placed on the sale by national Pharmaceutical council or bodies. However, high level of resistance of *E.coli* against nalidixic acid 16(80%), ceporex 12(60%) and Ampicilin 10(50%) obtained in this study could be due to indiscriminate use of these antibiotics such as overdosage, underdosage, irrelevant use and lack of strict adherence to prescriptions from the hospital.

The susceptibility of *E.coli* to ofloxacin and gentamycin; and resistance to nalidixic acid and ampicilin obtained in this research is in agreement with that of Manikandan and Amsath

(2014) in India.The most multiple antibiotic resistant *E.coli* detected in this study was found to be resistant to eight antibiotics out of a total of ten. This implies that the *E.coli* is highly pathogenic and could make treatment of diarrhoea very difficult; resulting in high cost of treatment and can easily lead to death of the patient.(Navia *et al.*,2004). However, the *E.coli* isolates resistant to 4 and 5 combinations of antibiotics could also be considered as having high resistant pattern and are highly pathogenic and of public health significance.

The MIC at 20.0mg/ml concentration of antibiotic (ofloxacin) was the lowest that showed no turbidity (no growth) in *E. coli* isolate number 5 and therefore, 20mg/ml was the MIC of the above mentioned isolate. 10mg/ml was the least concentration of antibiotic that tend to inhibit the growth of *E. coli* isolates number 4, 11 and 18 respectively and therefore and therefore 10.0mg/ml was the MIC of the above mentioned isolates. 5mg/ml was the least concentration of antibiotic (ofloxacin) that inhibit the growth of *E. coli* isolates number 1-3, 6-10, 12-17 and 19-20 and therefore, 5mg/ml was found to be the MIC of the above mention isolates. This might be the *E.coli* isolated from the diarrhoea stool of children attending Dutse General Hospital is of different strains. However, From the results of Minimum Bactericidal Concentration 20.0mg/ml was the least concentration of antibiotic (ofloxacin) that shows no growth on the plate of *E. coli* isolates number 3, 5, 9-10 and 15 and therefore, 20mg/ml was the MBC of the above mention isolates. 10mg/ml was the least concentration of antibiotic that shows no growth on the plate of *E. coli* isolates number 4, 6, 8, 13-14 and 16-20 respectably and therefore, 10.0mg/ml was the MBC of the above mentioned isolates. The least concentration of antibiotic ofloxacin (5mg/ml) that shows no growth on the plate of *E. coli* isolates number 1-2, 7 and 11-12 respectably and therefore, 5mg/ml was found to be the MBC of the mentioned isolates. This is an indication that the *E.coli* isolated in this research from the diarrhoea stool of children attending Dutse General Hospital might be of different strain.

CONCLUSIONS

Ofloxacin has the highest potency among other antibiotics used in this study and could be recommended for the treatment of diarrhoea caused by *E.coli*. Most of the *E.coli* isolates were found to be multiple antibiotic resistant indicating that they are pathogenic and might be the main etiological agent of diarrhoea in children attending Dutse General Hospital.

In the minimum inhibitory concentration and minimum bactericidal concentration, 20mg/ml, 10mg/ml and 5.0mg/ml of ofloxacin were found to be the least concentration that inhibit and kill the*E. coli* causing diarrhoeal infection.

It is therefore recommended that continuous study on antibiotic susceptibility testing should be encouraged to ascertain the potency of antibiotics used in the treatment of bacterial infection. Awareness should be created via mass media and seminar presentations on the implications of indiscriminate use of antibiotics. Appropriate measures should be taken by parents and care-givers to prevent the contamination of food and water by this enteropathogen. Genotypic determination of antibiotic resistance should be considered in further studies to be able todetect specific gene(s) responsible for the resistance.

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