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Research Article

SUSCEPTIBILITY PATTERN OF *E. COLI* ISOLATES OBTAINED FROM INFANTS DIARRHOEA ATTENDING SOME GENERAL HOSPITALS, KANO STATE, NIGERIA

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ABSTRACT
This study was conducted to determine the susceptibility pattern of *E. coli* isolates obtained from diarrhoeic infants stools attending some general hospitals in Kano state, Nigeria. Standard microbiological methods of culturing, microscopy and biochemical tests were used for the identification and characterization of the *E. coli* isolates from infant's stool samples collected. The results of susceptibility pattern of *E. coli* isolates on some commercial single discs antibiotics showed that the *E. coli* isolates are susceptible to ciprofloxcin 169 (83%), ceftriaxone 159 (78%), augmentin 155 (76%) and ofloxacin 153 (75%) antibiotics. The isolates are however, resistant to ampicillin 182 (89%), amoxicillin 170 (83%), sulphamethoxazole/trimethoprim 105 (51%) and tetracycline 94 (46%). Resistance patterns of *E. coli* isolates showed that ampicillin and amoxycillin has the highest single antibiotics disc resistance with 11 (12.5%) and 10 (11.4%) number of isolates respectively. Multiple antibiotic resistance pattern shows high frequency of occurrence in AMP, SXT, AML with 6 (6.82%), AMP, SXT with 5 (5.68%) and SXT, AML with 4 (4.55%) number of isolate with the patterns. Highest number of resistant pattern of 7 antibiotics in only one isolate with 1.14% occurrence resistance phenotype includes; OFX, AMP, SXT, CN, AML, CIP, TE was also obtained. No significant difference between the means of number resistance and susceptible organisms (P = 0.1). Proper diagnosis of diarrhoea and other microbial diseases should be carried out before drugs are been administer to patients.

Keywords: *Susceptibility, Antibiotics, Escherichia Coli, Diarrhoea, Infants, Kano*

INTRODUCTION
Plasmid-mediated antibiotics resistance is common in the treatment of *E. coli* diarrhoea, the use of antibiotics is in general only of minor importance. Rehydration is always the most important measure taken. Therapy with appropriate antibiotics can reduce the severity and duration of symptoms. Various means have been proposed and in use for the prevention of diarrhoea, including daily ingestion of bismuth subsalicylate suspension and regular doses of tetracyclines or other antimicrobial drugs such as ciprofloxacin or trimethoprim-sulfamethoxazole for limited period prophylaxis (Jawetz *et al.*, 2007). However, several traditional methods including use of herbs have been in practice for decades.
Escherichia coli is a common member of the normal flora of the large intestine. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals. Strains that acquire bacteriophage or plasmid Deoxyrhibonuecleic acid (DNA) encoding enterotoxins or invasion factors become virulent and can cause either plain, watery diarrhoea or inflammatory dysentery. These diseases are most familiar to Westerners as traveler's diarrhoea, but they are also major health problems in endemic countries, particularly among infants in developing nations. *Escherichia coli,* a facultative anaerobic gram-negative bacillus, is a major components of the normal intestinal flora and ubiquitous in the human environment.
First described in 1885, *E. coli* has become recognized as both a harmless commensal and a versatile pathogen. In contrast to the essential and beneficial role of most *E. coli* isolates in the human intestine, pathogenic *E. coli* are responsible for broad spectrum of human diseases. *E. coli* has emerged as an important cause of diarrheal illness, with diverse phenotypes and pathogenic mechanisms (Jawetz *et al.*,

2007). Hemolytic-uremic syndrome (HUS) is a potentially devastating consequence of enteric infection with specific *E. coli* strains.

Diarrhoea is usually defined as an increase number of stools of liquid or semi-liquid consistently passed during a twenty-four-hour period after the age of three months (Martines *et al.*, 1993). Diarrhoea infection occurs through water and food contamination with bacteria such as *Escherichia coli*, *Shigella* and other agents like *Vibrio cholerae*, *Compylobacter jejuni* and viruses such as Rotavirus; and protozoa such as *Giardia lambalia*, *Entamoeba histolytica* and *Cryptosporidium* (Okolocha and Umoh, 2002). It is acute when it lasts up to 21 days and chronic when it lasts beyond 21 days (Banerjee, 1988). Acute diarrheal diseases are one of the leading causes of childhood mortality and morbidity in the developing countries and a major contributor to malnutrition.

This research is aimed to determine the susceptibility pattern of *E. coli* isolates obtained from infants diarrhoea attending some general hospitals, Kano State, Nigeria.

MATERIALS AND METHODS

The Study Area

The study was carried out in Kano State, Nigeria located between latitudes and longitudes $12^{o}37'$N, $9^{o}29'$E and $9^{o}33'$S, $7^{o}34'$W respectively (Olofin *et al.*, 2008). The state has a total of 20,680 square kilometers and population of 9,383,682, 2006 census (National Population Commission-NPC, 2006). The population is predominantly Hausa-Fulani and other tribes from different parts of Nigeria are also found in various parts of the state. The people of Kano are mostly livestock and crop farmers and traders, though other professions also exist. The study area has a large population density especially in the metropolis local government. The pattern of the settlement is nucleated in nature. It is common to find a market, motor park, schools etc. next to hospitals or residential areas. Sanitation is poor with inadequate waste management, open dumped and illegal roadside dumping from residential and commercial areas remains a problem. Most of the water drainages and town ditches have been turned into solid waste disposal places. However, government is putting some effort into sanitation and provision of healthcare facilities, yet diseases such as diarrheoa, cholera, malaria, measles and other illnesses occurring as epidemic in the study area.

Sample Collection

Ethical committee clearance for collection of diarrhoeal sample from patients attending hospitals was obtained from the Kano State Health Services Management Board and the consent of the infants' care giver or parents was also solicited before collecting the diarrhoeal sample. A structural questionnaire was designed and administered to the mother/care giver of the infants (appendix IV). The questionnaire contained personal data of the infant, knowledge on the disease transmission, prevention and cure among other. Stool samples were collected from infants and children aged less than five years attending the selected general hospitals in the study area. The diarrhoeal samples were collected in accordance with the procedure described by Cheesbrough (2006). The diarrhoeal stool samples of at least 1g or 10 ml was collected in a clean, sterilized wide open mouth plastic container with lid-spoon, labeled and transported to the laboratory within 3-6 hours after collection in a box containing ice blocks as described by Atata *et al.*, (2003).

Isolation and Identification of Isolates

Growth Media Preparation

Plates of Eosin Methylene Blue (EMB) agar (L:S Biotech), Mueller Hinton agar (Oxiod) and Nutrient Agar (Oxoid) were prepared according to the manufacture's guide. The media was sterilized in an autoclave at 121^{o}C for 15 minutes. The media was then allowed to cool, then poured in to sterile Petri-dishes and allowed to solidify. Excess moisture was removed in a dryer for 15 minutes.

Isolation and Identification

An inoculum from the sample was streaked on Eosin Methylene Blue (EMB) agar plates and incubated overnight at 37^{o} C. Suspected *E. coli* colonies (bluish black with green metallic sheen colouration) from the EMB agar plate was streaked on Nutrient agar slant and refrigerated until further analysis. The

suspected isolates were subjected to gram staining, microscopy and biochemical tests (indole, lysine decarboxylate (LDC), citrate utilization, oxidase, urease, Voges-Proskauer (VP), lactose, etc.) as described by Cheesbrough (2006).

Preparation of Turbidity Standard

One per cent volume by volume (1% v/v) solution of hydrogen tetraoxosulphate vi acid (H_2SO_4) was prepared by adding 1 ml concentrated H_2SO_4 into 99ml of water. One per cent weight by volume (1% w/v) solution of barium chloride was also prepared by dissolving 0.5 g of dehydrated barium chloride in 50ml distilled water. Then, 0.6ml of the barium chloride solution was combined with 99.4 ml H_2SO_4 solution to yield 1.0 % w/v barium sulphate suspension. The turbid solution (Macfarland standard scale No. 6) formed was transferred into a test tube for comparison with turbid standard bacterial inocula preparation (Cheesbruogh, 2006).

Standardization of Inoculum

The isolates were activated by subculture into nutrient broth (Oxoid) and incubated at 30^{o}C for 6 hours. To make the standard inocolum, some quantity of the activated culture was transferred into a tube containing 2.0 ml normal saline (0.9% w/v) using sterile pipette, until the turbidity of the suspension matched the turbidity of the Macfarland scale number 1 standard (Cheesbruogh, 2006).

Susceptibility of E. coli Strains to Commercially Produced Antibiotics

One ml of the standard inoculum was evenly spread onto the surface of Muelar Hinton agar (Fluka BioChemica) plates in duplicates. This was incubated at 30^{o}C for 6 hours prior to the application of the antibiotic discs (Oxoid); ofloxacin, augmentin, sulphamethazole/trimethoprim, ciprofloxacin, tetracycline, ampicillin, amoxycillin, gentamicin and nitrofurantoin. The discs were placed firmly onto the surface of the solidified agar by means of sterile forceps (NCCLS, 1996). The plates were incubated at 37^{0} C for 24 hours. Diameters of the zones of inhibition were measured and recorded to the nearest mm in accordance with method adopted by Darwish *et al.*, (2002).

RESULTS AND DISCUSSION

Results

The results of susceptibility pattern of *E. coli* isolated from infants' diarrhoea attending some hospitals in Kano on some commercial single discs antibiotics is showed (Table 1). The result showed that the *E. coli* isolates are susceptible mostly to ciprofloxacin, ceftriaxone, augmentin and ofloxacin antibiotics with more numbers of susceptible with wider zones of inhibition of 169 (83%), 159 (78%), 155 (76%) and 153 (75%) respectively. The isolates are however, resistant to ampicillin, amoxycillin, sulphamethoxazole/trimethoprim and tetracycline with number resistance of 182 (89%), 170 (83%), 105 (51.47%) and 94 (46%) respectively. No significant difference between the means of number resistance and susceptible organisms (P = 0.1).

Table 2 shows resistance patterns of *E. coli* isolated from infants diarrhoea attending some hospitals in Kano state, Nigeria. Ampicillin and amoxycillin has the highest single antibiotics disc resistance with 11 (12.5%) and 10 (11.4%) number of isolates respectively. For the multiple antibiotic resistance pattern high frequency of occurrences were obtained in AMP, SXT, AML with 6 (6.82%), AMP, SXT with 5 (5.68%) and SXT, AML with 4 (4.55%) number of isolate having these patterns. Highest number of antibiotics resistant pattern of 7 antibiotics in only one isolate with 1.14% occurrence resistance phenotype includes; OFX, AMP, SXT, CN, AML, CIP, TE was also obtained.

Discussion

Resistance of *E. coli* due to transfer of plasmid and other factors could be responsible for the results of susceptibility pattern of the *E. coli* isolated from infants' diarrhoea attending some hospitals in Kano on commercial single discs antibiotics (Donnenberg *et al*., 1993; Cheetham and Katz, 1995). The susceptibility to ciprofloxacin, ceftriaxone, augmentin and ofloxacin antibiotics with number with larger zone of inhibition of 169 (83%), 159 (78%), 155 (76%) and 153 (75%) respectively could be due to the dispensing policy thus lack of their availability and high cost (Shakya *et al.*, 2013). The isolates resistance to ampicillin, amoxycillin, sulphamethoxazole/trimethoprim and tetracycline with number of 182 (89%),

170 (83%), 105 (51.47%) and 94 (46%) respectively, however, could results due to the frequent misuse and abuse.

Ampicillin and amoxicillin has the highest single antibiotics disc resistance with 11 (12.5%) and 10 (11.4%) number of isolates respectively. This could results be as a result of factors such as long term usage/ misuse, production of TEM-type β-lactamases often produced by most *E. coli* to hydrolyse penicillin and cephalosporins (Abalaka *et al.*, 2010). For the multiple antibiotic resistance pattern high frequency of occurrences were obtained in AMP, SXT, AML with 6 (2.94%), AMP, SXT with 5(2.45%) and SXT, AML with 4 (1.96%) number of isolate with the pattern. Highest number of antibiotics of 7 in only one isolate with 0.49% occurrence resistance phenotype includes; OFX, AMP, SXT, CN, AML, CIP, TE. This result indicates that *E. coli* resistance to antibiotics is increasing at alarming rate. However, the lower % in multiple antibiotics resistance as compared to the single discs indicates multiple/combination drugs treatment is the best means of treatment (Sule and Agbabiaka, 2008). Poor sanitation and personal hygiene, misuse and abuse of drugs could be the risk factors associated with the resistance and the pattern obtained.

Table 1: Susceptibility Pattern of *E. coli* Isolated from Infants Diarrhoea Attending some Hospitals in Kano on some Commercial Antibiotics Discs

Antibiotics	Abbreviation	Disc Potency (µg)	Number (%) Resistant Organisms	Number (%) Susceptible Organisms
Ceftriaxone (β -lactam-3rd Generation Cephalosporin)	CRO	5	45(22)	159(78)
Ampicillin (β-lactam-amino-penicillin)	AMP	10	182(89)	22(11)
Amoxycillin-clavulanic acid (β -lactam-β-lactamase inhibitor)	AMC	30	35(17)	155(76)
Amoxycillin (Penicillin, β-lactamase)	AML	10	170(83)	34(17)
Tetracycline (Tetracyciles)	TE	30	94(46)	110(54)
Ciprofloxacin (Fluoroquinine, 2nd generation)	CIP	5	49(24)	169(83)
Ofloxacin (Fluoroquinine, 2nd generation)	OFX	5	51(25)	153(75)
Gentamicin (Aminoglycoside)	CN	30	80(39)	124(61)
Nitrofurantoin (β -lactam-3rd Generation Cephalosporin)	F	200	74(36)	130(64)
Sulphamethoxazole/ Trimethoprim (Sulphonamide)	SXT	25	105(51.47)	99(48.53)

Table 2: Resistance Pattern of *E. coli* Isolated from Infants Diarrhoea Attending some Hospitals in Kano, Nigeria

Single Antibiotic Resistance		Multiple Antibiotic Resistance		
Number of Isolates (%)	**Resistance Phenotype**	**Number of Antibiotic Combination**	**Number of Isolates (%)**	**Resistance Phenotype with the Pattern**
11 (12.50)	AMP	2	1 (1.14)	F, SXT
10 (11.36)	AML		5 (5.68)	AMP, SXT
4 (4.55)	SXT		2 (2.27)	SXT AMC
2 (2.27)	TE		3 (3.41)	AMP, TE
1 (1.14)	F		4 (4.55)	SXT, AML
1 (1.14)	AME		1 (1.14)	AMP, CN
			3 (3.41)	AMP, AML
			2 (2.27)	TE, AMC
			1 (1.14)	AMP, AMC
			1 (1.14)	AML, TE
			1 (1.14)	CN, TE
		3	6 (6.82)	AMP, SXT, AML
			1 (1.14)	AMP, TE, AMC
			1 (1.14)	SXT, AML, CIP
			1 (1.14)	F, AML, CRO
			2 (2.27)	AMP, SXT, TE
			1 (1.14)	SXT, AML, AMC
			2 (2.27)	AMP, AML, TE
			2 (2.27)	AML, TE, AMC
			1 (1.14)	CN, AML, AMC
			1 (1.14)	AMP, AML, AMC
			1 (1.14)	OFX, SXT, CN
		4	1 (1.14)	AMP, CN, TE, AMC
			1 (1.14)	F, AMP, AML, CIP
			1 (1.14)	AMP, SXT, AML, CIP
			1 (1.14)	AMP, SXT, AML, AMC
			1 (1.14)	AMP, AML, TE, AMC
			2 (2.27)	AMP, SXT, AML, TE
			1 (1.14)	F, AMP, CN, AMC
		5	3 (3.41)	AMP,SXT, AML, TE, AMC
			1 (1.14)	F, SXT, AML, TE, AMC
			1 (1.14)	OFX, AMP, AML, TE, AMC
		6	1 (1.14)	OFX, SXT, CN, AML, CIP, TE
			1 (1.14)	OFX, AMP, SXT, CN, AML, TE
		7	1 (1.14)	OFX, AMP, SXT, CN, AML, CIP, TE

Key: *OFX= Ofloxacin, F= Nitrofrantion, AMP= Ampicillin, SXT= Sulphamethoxazole-Trimethoprim (co-Tromathoprim), CN= Gentamycin, AML= Amoxycilline, CIP= Ciprofloxacine, TE= Tetracycline, AMC= Amoxicillin and Clavulanic Acid (Agumentin), CRO= Ceftriazone*

Conclusion and Recommendations

The *E. coli* isolates were highly resistance to β-lactamase and salphonamide antibiotics. However, high susceptibility to fluoroquinine, cephalosporin, amoxicillin-clavulanic acid (Augmentin) and ofloxacin is observed. The isolates also revealed both single and multiple antibiotics resistance pattern.

It is recommended that proper and strict dispensing policy of antibiotics should practice in the state and the nation in general. Diagnosis of diarrhoea and other microbial diseases should be carried out before drugs are administered to patients.

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