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Full Length Research Paper

Diarrheagenic *Escherichia coli* (DEC): Prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agents

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The prevalence of diarrheagenic *Escherichia coli* both in an ambulatory patients passing out loose stools with or without blood and/or mucus in Anua General Hospital, University of Uyo Teaching Hospital and University of Uyo Health Centre from June to September, 2008 were determined using standard microbiological techniques. Susceptibility to seven different conventional and commonly available chemotherapeutic drugs/antibiotics: ampicillin, chloramphenicol, ciprofloxacin, gentamycin, tetracycline, cephalothin and ofloxacin were assessed using a disc diffusion technique (DDT). The macroscopic analysis of the stool samples showed that 31 of the 100 cases (31%) were diarrhea bloody and 33% mucoid. Sixty-nine diarrheagenic *E. coli* were isolated from 100 stool samples collected and were more prevalent in females (69.4%) than in males (30.6%). The observed percentage prevalence of diarrheagenic *E. coli* among the age groups (in years) 1 -15, 16 - 30, 31 - 45, 46 - 60 and 61 and above were 95, 80, 55, 70 and 45%, respectively. The results of antibiotic susceptibility showed that the *E. coli* were highly resistant to ampicillin (73.9%), tetracycline (75.4%) and gentamycin (68.1%), and moderately resistant to chloramphenicol (46.4 %) and cephalothin (43.5%), but highly sensitive to ciprofloxacin (71.0%) and ofloxacin (66.7%). The findings of this study showed ciprofloxacin and ofloxacin to be drugs of choice for the treatment of diarrheagenic *E. coli,* while ampicillin, tetracycline and gentamycin should not be used without first performing culture and sensitivity tests.

Key words: Diarrheagenic, *Escherichia coli,* prevalence, chemotherapy, susceptibility.

INTRODUCTION

Escherichia coli are common members of the normal flora of the human intestine (Nataro and Kaper, 1998; Yah et al., 2006). Strains of *E. coli* that acquire bacterio-phage, plasmid DNA encoding enterotoxin or invasion factors become virulent. This virulence increases the ability of *E. coli* to adapt to new niches to cause a broad spectrum of diseases such as urinary tract infections and nosocomial infections resulting in either a plain, watery diarrhea or inflammatory dysentery. *E. coli* are prominent members of Enterobacteriaceae and are widely distribu-ted in nature, they are present in the intestinal tract of man and animals, and in water and soil (Nataro et al., 1987; Smith et al., 2003). Diarrhea caused by *E. coli* infection is one of the major public health concerns in many developing countries and has contributed exceedingly to morbidity and mortality, and also the associated increase in health costs (Adachi et al., 2001; Ogata et al., 2002; Robins-Browne and Hartland, 2002). *E. coli* have also been reported to be the leading cause of diarrhea-causing diseases in addition to bacterial pathogens such as *Salmonella* spp, *Shigella* spp, *Yersinia* spp, *Vibrio* spp, *Campylobacter* spp, *Enterobacter* spp, *Citrobacter* spp, *Proteus* spp, and parasitic pathogens such as *Entamoeba histolytica*, and *Giardia lamblia* in developing countries (Su and Brandt 1995; Smith et al., 2003; Prescott et al., 2008). Individuals who are debilitated or have other predisposing factors are at a much higher risk of infection than healthy persons. Strains of *E. coli* can be classified as commensal, intestinal pathogenic or extra intestinal pathogenic *E. coli* (ExPEC). *E. coli* pathotypes responsi-

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responsible for intestinal infections are enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli (EIEC),* enteropathogenic *E. coli* (EPEC), or enterotoxigenic *E. coli* (ETEC) (Rademaker et al., 1993; Paton and Paton 1998; Yah et al., 2006). Consumption of faecally contaminated water is an important route of transmission of diarrheagenic pathogens especially *E. coli,* in many regions of the world lacking infrastructure to guarantee water quality and safe management of human waste (Swerdlow et al., 1992) . *E. coli* is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics (Winokur et al., 2001; Miranda et al., 2004; Poppe et al., 2005). Antimicrobial resistance of *E. coli* has played an important role in clinical infectious diseases (Winokur et al., 2001) . Thus, the aim of this investigation was to determine the prevalence of diarrheagenic *E. coli* both in ambulatory patients in Uyo City and assess their susceptibility to different conventional and commonly available chemotherapeutic agents/antibiotics.

MATERIALS AND METHODS Collection

and processing of samples

Stool samples from diarrheagenic patients (patients passing out at least three loose stools in a 24 h period accompanied by symptoms such as nausea and/or abdominal cramp and/or fever (>38°C) were collected between July and September, 2008 for a prospective study of three different hospitals in Uyo City: Anua General Hospital, University of Uyo Teaching Hospital and University of Uyo Health Centre. Stool samples from patients who had not received antibiotic treatment at the time of investigation were collected aseptically using clean, sterile wide-mouth containers and taken to the Microbiology Laboratory of University of Uyo for bacterial analyses within 1 - 4 h of collection. Stool samples that could not be analyzed immediately were refrigerated at 4°C for less than 24 h. Characterization and identification of *E. coli* cultures were made on the basis of morphology, cultural characteristics, and biochemical reactions. All the stool samples were cultured into MacConkey agar (MCA) for primary isolation of common intestinal pathogens and incubated at 37°C for 24 h. All colonies on MacConkey agar plates suspected to be *E. coli* (lactose fermenter, non- mucoid, 2 - 3 mm diameter, circular, smooth and convex) were streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Green metallic sheen colonies positive for *E. coli* were further sub-cultured onto nutrient agar and incubated for another 24 h. The cultures on nutrient agar plates were subjected to Gram's-staining, motility, urease production, glucose, oxidase, sucrose, mannitol, lactose, indole, Voges proskauer, and citrate utilization tests. All Gram-negative, rod-shaped, motile, indole-negative, urease-negative isolates that produced acid on Triple Sugar Iron agar slants were identified as species of the genus *E. coli* with reference to Cowan and Steel (1985); Fawole and Oso (1988); Cheesbrough (2004).

Antibiotic sensitivity testing

In vitro susceptibility of the *E. coli* to seven different antibiotics was determined using a disk-diffusion technique (NCCLS, 2004). Sterile Petri dishes of Mueller Hinton agar were prepared according to the manufacturer's specification. 0.1 ml of *E. coli* was seeded into each of the Petri dishes containing Mueller-Hinton agar and were allowed to stand for 45 min to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: gentamycin (Gen, 10 g), ofloxacin (Ofl, 30 g), ampicillin (Amp, 10 g), tetracycline (Tet, 30 g), cephalothin (Cep, 30 g), ciprofloxacin (Cip, 5 g), chloramphenicol (Crp, 30 g) (Oxoid, UK) were aseptically placed on the surfaces of the sensitivity agar plates and these were incubated for 18 - 24 h at 37°C. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters. The interpretation of the measurement as sensitive, intermediate and resistant was made according to the manufacturer's standard zone size interpretive manual which were as follows: ofloxacin (S 21, I =16 - 20 and R 15), ciprofloxacin (S 21, I = 16 - 20 and R 15), gentamicin (S 15, I = 13 - 14 and R 12), ampicillin (S 17, I = 14 - 16 and R 13), chloramphenicol (S 18, I = 13 - 17 and R 12), cephalothin (S 18, I =15 - 17 and R 14) and tetracycline (S

19, I = 15 - 18 and R 4) where S = sensitivity, I = intermediate and R = resistance.

The intermediate readings were considered as sensitive for the assessment of the data. The choice of the above antibiotics used was based on local availability.

RESULTS

A total of sixty-nine (69) diarrheagenic *E. coli* were isolated from 100 stool samples collected from three different hospitals which translated to 69% of all samples being positive for *E. coli* during the study period (Tables 1 and 2). The macroscopic analysis of the stool samples showed that 31 of the 100 cases (31%) were diarrhea bloody and 33% mucoid. Forty-four of the subjects were male and 56% were female. Table 3 shows that diarrheagenic *E. coli* were more prevalent in females (69.6%) than in males (30.4%) and the observed percentage prevalence of diarrheagenic *E. coli* among the age groups (in years) 1 - 15, 16 - 30, 31 - 45, 46 - 60 and 61 and above were 95, 80, 55, 70 and 45%, respectively (Table 2).

The antimicrobial sensitivity tests of diarrheagenic *E. coli* to seven antibiotics by a disc diffusion method is shown in Tables 4 and 5. The results showed that 66.7 - 71.0% of diarrheagenic *E. coli* were found to be highly sensitive against ofloxacin (15 mm diameter) and ciprofloxacin (16 mm diameter), while 43.5 and 46.4% were found to be moderately resistant against cephalothin (18 mm diameter), and chloramphenicol (12 mm diameter), respectively. The results of the antibiotic susceptibility also showed most of the isolates to be highly resistant to the antibiotics ampicillin (73.9%), tetracycline (75.4%) and gentamycin (68.1%) with inhibitory zones ranging from 0 to 10 mm. Multidrug resistance (2 - 6) to antibiotics was observed in 49 cases (71.0%) and the major resistance profile was ampicillin-gentamycin-tetracycline.

DISCUSSION

E. coli infection is one of the major public health problems in many developing countries and has contributed exceedingly to morbidity, mortality and increased health costs (Adachi et al., 2001; Ogata et al., 2002; Robins-

Table 1. Macroscopic and diarrhea status of *Escherichia coli.*

Nature of Stool Sample	No. Positive for *E. coli*	No. Negative for *E. coli*	Total
Watery	33	16	49
Watery + Bloody	11	07	18
Watery + Mucoid	16	04	20
Watery + Bloody + Mucoid	09	04	13
Total	69	31	100

Table 2. Age-specific prevalence of diarrheagenic *Escherichia coli*.

Age (Years)	No. of Samples	Occurrence of *E. coli* Isolated	Percentage of Isolates
0-15	20	19	95
16-30	20	16	80
31-45	20	11	55
46-60	20	14	70
61and above	20	09	45
Total	100	69	69

Table 3. Sex-specific prevalence of diarrheagenic *Escherichia coli.*

Sex	No. of Samples	No. of Isolates	Percentage of Isolates
Male	44	21	30.4
Female	56	48	69.6
Total	100	69	100.0

Browne and Hartland, 2002)

Our results reveal the prevalence of diarrheagenic *E. coli* among the age groups (in years) 1 - 15, 16 - 30, 31 - 45, 46 - 60 and 61 and above to be 95, 80, 55, 70 and 45%, respectively. The prevalence of diarrheagenic *E. coli* among females was greater than that of males and these results are in conformity with those obtained by Diame et al. (1990) and Lothar et al. (1998).

In recent years, antibiotic resistance of diarrheagenic pathogens has reached alarming proportions worldwide. The misuse of antibiotics has been found to be the most important selecting force in bacterial antibiotic resistance (Okeke et al., 1999; Yah and Eghafona, 2007; Akinjogunla et al., 2008). Antibiotic resistance of *E. coli* to ampicillin, gentamycin and chloramphenicol recorded in this study are similar to those obtained by Okeke et al. (2000); Okoli et al. (2002). The resistance of some diarrheagenic *E. coli* in this study to at least one of the seven antibiotics tested especially tetracycline, ampicillin and gentamycin could be a result of the routine and uncontrolled use in patients. Also, regarding the sensitivity pattern, this study showed that ofloxacin and ciprofloxacin were effective against diarrheagenic *E. coli* and is in agreement with previous findings (Yah and Eghafona, 2007). The low level of resistance of these quinolones may be because they are relatively new antibiotics and are also more expensive than tetracycline, ampicillin and gentamycin. There is evidence indicating that tetracycline survives longer in the environment than other antibiotics which may be critical in maintaining the level of tetracycline resistance at a high level (Yah and Eghafona, 2007)

Monitoring drug resistance patterns of *E. coli* will give vital clues to clinicians regarding therapeutic regimes to be adopted against individual cases and will be an important tool to devise a comprehensive chemo-prophylaxis.

Conclusion

The development of new antibiotics may offer a short-term solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation, should be emphasized.

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Table 4. Occurrence and percentage of antibiotic resistant diarrheagenic *Escherichia coli.*

Hospital	No. of Samples Collected	No. of *E. coli* Isolated	No. (%) Resistant to CIP	No. (%) Resistant to GEN	No. (%) Resistant to CRP	No. (%) Resistant to AMP	No. (%) Resistant to OFL	No. (%) Resistant to TET	No. (%) Resistant to CEP
AGH	33	22	6 (27.3)	14 (63.6)	10 (45.5)	16 (72.7)	7 (31.8)	17 (77.3)	11 (50.0)
UUTH	40	29	9 (31.0)	20 (68.9)	14 (48.3)	22 (75.9)	11 (37.9)	20 (69.0)	11 (37.9)
UUHC	27	18	5 (27.8)	13 (72.2)	8 (44.4)	13 (72.2)	5 (27.8)	15 (83.3)	8 (44.4)
Total	100	69	20 (30.0)	47 (68.1)	32 (46.4)	51 (73.9)	23 (33.3)	52 (75.4)	30 (43.5)

Keys: AGH: Anua General Hospital; UUTH: University of Uyo Teaching Hospital; UUHC: University of Uyo Health Centre; CIP: Ciprofloxacin; GEN: Gentamycin, CRP: Chloramphenicol; AMP: Ampicillin; OFL: Ofloxacin; TET: Tetracycline; CEP: Cephalothin.

Table 5. Occurrence and percentage of antibiotic sensitive diarrheagenic *Escherichia coli.*

Hospital	No. of Samples Collected	No. of *E. coli* Isolated	No. (%) Sensitive to CIP	No. (%) Sensitive to GEN	No. (%) Sensitive to CRP	No. (%) Sensitive to AMP	No. (%) Sensitive to OFL	No. (%) Sensitive to TET	No. (%) Sensitive to CEP
AGH	33	22	16	8	12	6	15	5	11
UUTH	40	29	(72.7)	(36.4)	(54.5)	(27.3)	(68.2)	(22.7)	(50.0)
UUHC	27	18	20	9	15	7	18	9	18
Total	100	69	(69.0)	(31.0)	(51.7)	(24.1)	(62.1)	(31.0)	(62.1)
			13 (72.2)	5 (27.8)	10 (55.6)	5 (27.8)	13 (72.2)	3 (16.7)	10 (55.6)
			49 (71.0)	22 (31.9)	37 (53.6)	18 (26.1)	46 (66.7)	17 (24.6)	39 (56.5)

Keys: AGH: Anua General Hospital; UUTH: University of Uyo Teaching Hospital; UUHC: University of Uyo Health Centre; CIP: Ciprofloxacin; GEN: Gentamycin, CRP: Chloramphenicol; AMP: Ampicillin; OFL: Ofloxacin; TET: Tetracycline; CEP: Cephalothin.

of specimens

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