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Determination of fecal carriage rate of Fluoroquinolone Resistant *E. coli* in Hospital and community Settings of Damaturu, Yobe State, Nigeria

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Abstract

The level of resistance among Enterobacteriaceae is a concern for global health, and of particular concern is the spread of fecal carriage rates of fluoroquinolone resistance. This research aimed to assess the rates of fecal carriage of fluoroquinolone-resistant E. coli in both hospital and community environments in Damaturu. This study involved random selection of total of 200 participants, comprising 100 hospitalized patients and 100 individuals from the community. A stool sample was collected from every participant. A questionnaire was administered to assess potential risk factors associated with the colonization of resistant bacteria. Ciprofloxacin (CIP) at a concentration of 1 mg/L was used to screen for the colonization of CIP-resistant E. coli using phenotypic and confirmatory techniques. The bacterial isolates were further subjected to a disc diffusion test to confirm resistance. The overall CIP-resistant E. coli rate was 41.5% (83/200). The 19- to 30-year age group included 83 participants, accounting for 41.5% of the total. Among individuals aged 31 years and older, there were 117 participants, accounting for 58.5%. The total comprised 108 males, which is 54.0%, and 92 females, making up 46.0%. In the study population, 58 (29.0%) participants reported having gastrointestinal system issues (diarrhea) at the time of sample collection. The history of antibiotic use among participants in the year preceding the study was 132 (66.0%). There was significant association between gender and CIP-resistant E. coli. A higher prevalence of fecal carriage of fluoroquinolone-resistant E. coli was reported in hospitalized groups (77, 60.0%) than in community groups (51, 39.8%). This research suggests that intestinal colonization by fluoroquinolone-resistant E. coli can occur in both hospital and community settings. Consequently, it is essential to implement control measures to prevent the spread of resistant bacteria.

Key words: E. coli, fecal carriage, Antibiotic resistance, Enterobacteriaceae, Fluoroquinolone

INTRODUCTION

The Enterobacteriaceae family of Gram-negative bacteria was first described by Rahn in 1936 (Walker *et al.*, 2018). It currently comprises over 30 genera and more than 100 species, all of which are bacilli with a typical length of 1–5 nm (Walker *et al.*, 2018). As their name suggests, these microorganisms are frequently found in the gastrointestinal tract (GIT) where they are essential for maintaining human health and are also a natural component of the flora in animals (Walker *et al.*, 2018). It is noteworthy that Enterobacteriaceae are frequently dispersed throughout the environment.

Escherichia coli is a member of the Enterobacteriaceae, a facultative anaerobe that is a normal inhabitant of the human and animal gastrointestinal tract (Bradford, 2001; Kuskucu *et al.*, 2016). While most strains are commensal, some are pathogenic, causing a range of infections, including urinary tract infections (UTIs), bloodstream infections, and gastroenteritis (Bradford, 2001). In addition, *E coli* can occasionally cause diarrhea when consumed through tainted food or water (Walker *et al.*, 2018). Some of the bacteria that cause diarrhea are enteropathogenic *E. coli* (EPEC), which causes diarrhea in infants; enterotoxigenic *E.coli* (ETEC), which causes diarrhea that mimics cholera; and other bacteria that produce diarrhea that is linked to travel (Kuskucu *et al.*, 2016). Enteroinvasive *E. Coli* (EIEC) is another variety that bears a striking resemblance to *Shigella* species in terms of disease, specifically dysentery (Kuskucu *et al.*, 2016). The bacteria known as

enterohemorrhagic *E. coli* (EHEC), specifically serotype O157:H7, is the primary cause of hemorrhagic colitis and bloody diarrhea (Kuskucu *et al.*, 2016).

Antibiotic resistance has emerged as a worldwide concern that develops rapidly and poses a significant threat to the effectiveness of antibiotics in treating bacterial infections (Ventola, 2015). Despite the initial effectiveness of antibiotics in treating bacterial infections decades ago, resistance emerged not long after the discovery of these agents (Da Silva *et al.*, 2007; Ventola, 2015). Fluoroquinolones have been tagged as a "five star" antibiotic and have helped in the treatment of life-threatening infections; however, the emergence of resistance has reversed the success achieved (Claeys *et al.*, 2018). Fluoroquinolones have been used as a five-star drug to treat many bacterial infections, some of which are life-threatening (Claeys *et al.*, 2018). Furthermore, fluoroquinolones, in conjunction with other antibacterial agents, have been widely utilized in veterinary medicine, either for therapeutic purposes or as growth enhancers. (Claeys, *et al.* 2018).

Fluoroquinolone-resistant *Escherichia coli* (FQ-*E. coli*) represents a global health concern. FQ-resistant *Escherichia coli* has been reported in numerous studies, with several associated risk factors, including previous antibiotic use (Steensel et al., 2012; Reuland et al., 2016; Emrah *et al.*, 2019). Benameur *et al.* (2018) reported the isolation of fluoroquinolone-resistant strains of Enterobacteriaceae and the identification of qnrS in *E. coli*. A four-year study conducted in western Algeria from 2010 to 2014 revealed significant frequencies of fluoroquinolone-resistant Enterobactericeae being carried in feces. In a particular study, 233 of the 253 (92.09%) Enterobacteriaceae strains that were identified exhibited multidrug resistance. This indicates that the prevalence of fecal carriage of fluoroquinolone-resistant Enterobacteriaceae is significantly higher (Benameur *et al.* 2018). Similarly, Saksena *et al.* (2018) *reported a* high colonization rate of antibiotic-resistant *E. coli*. Out of 100 newborns, 343 Enterobacteriaceae were isolated; of these, 58% carried at least one Enterobacteriaceae member, primarily *E. coli*, and 60% of them were fully resistant to nalidixic acid.

Fecal carriage rates of FQ-resistant *E. coli* are reported worldwide; however, such records are limited in this study area. Therefore, the present study aimed to determine the fecal carriage rates of FQ-resistant *E. coli* in Damaturu, Yobe State, Nigeria. Furthermore, the possible risk factors associated with intestinal colonization of FQ-resistant *E. coli* were assessed.

MATERIALS AND METHODS

Study design and subjects involved

A random fecal Sample collection was conducted between January 2023 and July 2023. A total of 200 participants were included in this study for equal distribution. A total of 100 hospitalized patients participated and stayed in the General Sani Abatcha State Specialist Hospital, Damaturu, Yobe State, for at least 3 days. A total of 100 community groups consisted of people who had no history of hospitalization in the year preceding the study's commencement.

Inclusion criteria

All study participants were above 18 years old.

Collection of Samples and Data

A total of 200 fecal samples were collected for the study. A simple questionnaire was administered to ascertain the variables associated with intestinal colonization of bacteria resistant to antibiotics throughout the sample collection process. First, basic demographic data were collected, including age, marital status, and gender. Other questions were presence or absence of any gastrointestinal (diarrhea) issues they may have experienced. Additionally, data on hospitalizations and antibiotic use during the year preceding the study were collected. The patients also disclosed details on the length of their hospital stay, their time spent in the intensive care unit, and the use of antibiotics during their most recent hospital stay.

Initial Screening of Fluoroquinolone-Resistant *Enterobacteriaceae*

The protocol involved suspending 200 mg of fecal specimens in 2 mL of sterile saline. Every sample was inoculated on two plates (EMB medium) supplemented with ciprofloxacin (CIP) from Merck, Germany. For every sample, a control plate was used to measure the amount of bacteria growing, as described by Emrah *et al.* (2019). For the purpose of screening for fluoroquinolone resistance, each sample was placed in a medium containing 1 mg/L of the antibiotic ciprofloxacin (CIP) from Sigma, USA.

After that, the medium was supplemented with 1 mg/L CIP. Following the inoculation of an aliquot of each stool suspension on media containing antibiotics as well as controls, the plates were incubated for 24 hours at 37 °C. The plates were assessed for bacterial growth following the incubation time. Colonies of bacteria that grew on media containing antibiotics were stored for identification and confirmation at -20°C in stock media, which consisted of nutritional broth in microcentrifuge tubes with 15% glycerol.

Identification and Phenotypic Confirmatory Tests for the Ciprofloxacin-Resistant *Enterobacteriaceae*

After being inoculated into EMB, the samples were incubated at 37 °C for 24 hours. Suspensions of 0.5 McFarland standard turbidity were made following the development of bacteria. Using the disc diffusion test, bacterial suspensions were inoculated onto Mueller-Hinton media (Merck, Germany) to confirm fluoroquinolone resistance. Each plate contained five antibiotic discs (Bioanalyse, Turkey). These were CIP (5 µg), ofloxacin (OFX, 5 µg), norfloxacin (NOR, 10 µg), levofloxacin (LVX, 5 µg), and gemifloxacin (GEM, 5 µg). Zones of inhibition of 15 mm or less for GEM and CIP; 12 mm or less for NOR and OFX; and 13 mm or less for LVX antibiotic discs were recorded as resistant (Clinical Laboratory Standard Institute, CLSI, 2018).

Statistical Analysis

Variables in the questionnaire were analyzed using IBM SPSS Statistics package for Macintosh (Demo version 22.0; Armonk, NY: IBM Corp.). Significance level was accepted to be 0.05. X^2 was used to determine whether there was an association between gender and age group, and the resistance of antibiotics.

RESULTS

General Characteristics of the Study Participants

The total number of participants in the study comprised 200 participants; 100 from General Sani Abatcha State Specialist Hospital and 100 participants from the community setting. The age group ranged from 19 to 30 years and consisted of 83 (41.5%) participants. In the 31 years and above age group, there were 117 (58.5%) participants. There were 108 (54.0%) males and 92 (46.0%) females. In the study population, 58 (29.0%) participants reported having GIS (diarrhea) at the time of sample collection. The history of antibiotic use among participants in the year preceding the study was 132 (66.0%).

Fecal Carriage Rate of CIP-RE Isolates

The intestinal colonization rate of CIP-RE was 64.0% (n=128/200). In the Hospitalized group, 77 (60.0%) represented CIP-RE colonization, while 51 (39.8%) people in the community group had the same colonization (Table 1).

Table 1. Fecal carriage rate of CIP-RE positive

Participants	CIP-RE positive n128 (%)
Hospitalized	77 (60.0)
Community	51 (39.8)
Total	128 (64.0)

Resistance Rates of CIP-RE Isolates Against Other Fluoroquinolones

Among CIP-resistant isolates, resistance rates to OFX, NOR, LVX, and GEM were found to be 96.1% (n=123/128), 97.7% (n=125/128), 96.9% (n=124/128), and 98.4% (n=126/128),

Ciprofloxacine resistant to ofloxacine is 123 resistant out of 128 positive samples, ciprofloxacine positive 128 to norfloxacine, the resistant was 125 out of 128, representing 97.7%, ciprofloxacine agains levofloxacine, 128 resistant CIP-RE isolate agains levofloxacine the resistant 124 out of 128 representing 96.9%, ciprofloxacine resistant againe gemifloxacine, 128 ciprofloxacine resistant agains gemifloxacine is 126 isolate out of 128 samples representing 98.4% (Table 2).

Correlation of intestinal colonization of CIP-RE with demographic and socioeconomic factors

There was a statistically significant difference between the age of the study population and intestinal colonization (p = 0.022). No statistical association between intestinal colonization and gender (Table 3).

Between the age and gender groups, ranging from 19 to 30 years old, we collected 153 samples, among which 78 are positive, representing 51% of the samples. From the age of 31 years and above, we find that 75 samples out of 153 are positive, representing 49% of the samples.

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Correlation of intestinal colonization of CIP-RE with epidemiological factors

The fecal carriage in relation to fluoroquinolone with epidemiological factors is presented (Table 4).

Table 2. Resistance patterns of CIP-resistant isolates (n=128) against other fluoroquinolones.

	Ofloxacin	Norfloxacin	Levofloxacin	Gemifloxacin
Resistant	123 (96.1)	125(97.7)	124 (96.9)	126(98.4)
Intermediate	4 (3.1)	2 (2.3)	1 (0.8)	2 (1.6)
Susceptible	1 (0.8)	0 (0.0)	3 (2.3)	0 (0.0)
Total	128 (100.0)	128 (100.0)	128 (100.0)	128 (100.0)

Table 3. Correlation of intestinal colonization of CIP-RE with demographic parameters

Epidemiological factors	CIP-RE	
	Positive n/N (%)	*p* value
Age(years)		
19-30	78/153 (51)	0.022
31 and above	75/153 (49)	
Total	153/153 (100)	
Gender		
Male	88/164 (53.7)	0.544
Female	76/164 (46.3)	
Total	164/164 (100)	

Table 4. X^2 square of intestinal colonization of CIP-RE with epidemiological factors in the study group (N=128) (*The period covers the last 1 year before the study)

Epidemiological factors	CIP-RE	
	Positive n/N (%)	*p* value
Presence of any GIS at the time of sample collection		
Yes	58/128 (45.3)	0.388
No	70/128 (54.7)	
Total	128/128 (100)	
History of antibiotic use*		
Yes	85/128 (66.4)	0.884
No	43/128 (33.6)	
Total	128/128 (100)	

DISCUSSION

Fluoroquinolones have been excellent in helping to treat life-threatening infections. This study focuses on the fecal carriage rates of Fluoroquinolone-resistant *E. coli*. Records on the Resistance rates of *Enterobacteriaceae* are well published (Kotb *et al.*, 2019; Nakano *et al.*, 2019; Trautner, 2018), however, the rates of intestinal colonization by fluoroquinolone-resistant Enterobacteriaceae in both hospitalized patients and the community population in Damaturu are not clearly defined. Therefore, it is necessary to study the resistance rates of *Enterobacteriaceae* against different antibiotics, especially fluoroquinolones, which is an important class of antibiotics used to treat high-priority pathogens (Breijyeh *et al.*, 2020).

The development of resistance by bacteria has continued to threaten global public health (Shaikh *et al.*, 2015). The persistent use and misuse of drugs in the treatment of bacterial infections have been recognized as one of the

drivers of the emergence of antibiotic resistance (Rather *et al.*, 2017). A study that includes data from both community and hospital settings must be reported, as numerous studies have been conducted in both settings, producing varying results on the risk variables and their relationship to resistance. The bacterial species among CIP-RE isolates were identified. Taha *et al.* (2019) reported 37% *E. coli* isolates among CIP-resistant *Enterobacteriaceae*, while Schulz et al. (2016) reported 52% *E. coli* isolates in CIP-RE. The rates documented by the two studies are lower than the percentage obtained in this study.

The resistance rates of CIP-RE isolates to OFX, NOR, LVX and GEM were 96.1%, 97.7%, 96.9%, and 98.4%, respectively. Higher susceptibility of LVX when compared to OFX and NOR may be due to the improved efficacy as a 3rd generation fluoroquinolone (Idowu and Schweizer, 2017). Some studies, such as Ruh et al. (2016), reported lower resistance rates compared to this study. This could be due to variability in resistance across different geographical locations.

The result of the current study is consistent with the study by Duplessis *et al.* (2012), which found a statistical correlation between age and fluoroquinolone resistance. Additionally, it suggested that age might be a predictive factor in revealing the history of antibiotic usage and hospitalization. Furthermore, Sadigov *et al.* (2017) *reported a high correlation between older age and* antibiotic resistance in a community-based study in Azerbaijan.

Furthermore, the research conducted by de Lastours et al. (2014) indicated a significant association between gender and fluoroquinolone resistance. Additionally, another study observed that the patterns of antibiotic resistance, including penicillin and quinolone, were comparable in both males and females; notably, a very high level of resistance was identified in E. coli (Singh *et al.*, 2018). However, some studies have indicated that females generally exhibit greater antibiotic resistance compared to their male counterparts. This phenomenon may be attributed to certain infections that disproportionately affect females, such as urinary tract infections (Dolk et al., 2018; Smith et al., 2018). Moreover, Singh *et al.* (2018) demonstrated a statistical correlation between the education levels of participants and their antibiotic resistance.In this study, there was no correlation between the use of GIS at the time of stool sample collection and the fecal carriage of CIP-RE. This is also contrary to the recent studies of Abdallah *et al.* (2017) and Reuland *et al.* (2016), which showed statistical significance in community-onset gastrointestinal complaints.

The key drivers of antibiotic resistance development are the uncontrolled use of antibiotics, along with other factors (Ventola, 2015). However, Caudell et al. (2018) found no correlation between the use of antibiotics and antimicrobial resistance in both humans and livestock in Tanzania. In a separate investigation by Williamson *et al.* (2018), a notable reduction in antibiotic resistance was observed during a specific period in Australia; however, this was followed by a rise in prevalence. This increase may be attributed to the ongoing use of antibiotics within the population. Antibiotic resistance is primarily selected in community environments through exposure to antibiotics, and fluoroquinolones have been investigated as potential alternatives to treatment. In cases of urinary tract infections (UTI), exposure to fluoroquinolones was observed to significantly decrease Enterobacteriaceae; however, concurrently, this resulted in colonization with CIP-resistant Enterobacteriaceae in UTI patients (Stewardson *et al.*, 2018).

CONCLUSION

This study highlighted the fecal carriage rates of fluoroquinolone-resistant E. coli and possible risk factors associated with such as antibiotic-taking history and history of diarrhea and GIS disorder. The fecal carriage rate of CIP-RE was discovered in this investigation, and other epidemiological factors were also determined. The CIP-RE isolates exhibited resistance rates in correlation with other members of flouroquinolones such as OFX, NOR, LVX, and GEM. Based on this study, further research and surveillance studies using molecular techniques should be conducted in the study area to address the issue of Fluoroquinolone Resistance.

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