Prevalence of *E. coli* O157:H7 in the Urinary Tract of Apparently Healthy Students of a Tertiary Institution in Benin City, Nigeria

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ABSTRACT

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world and a major causative agent of severe Urinary Tract Infections. The isolation and prevalence of *Escherichia coli* O157:H7 was conducted according to standard techniques. A total of 150 urine samples were collected from apparently healthy students of University of Benin, Benin City and screened based on culture and biochemical. Also multiple antibiotic susceptibility was evaluated using Kirby Buer disc diffusion technique. *Escherichia coli (34.5%)*, *Staphylococcus aureus (31.3%)*, *Proteus mirabilis* (12.2%) and *Klebsiella spp (22.0%)* were isolated, also 17 (11.33%) were enumerated as *E. coli 0157:H7.* It was observed that all the 17 isolates of *E. coli 0157:H7* were resistant to Ciprofloxacin (5µg) and Ceftazidime (30µg) and all the isolates had multiple antibiotic resistant index of >2. Hence the need for antibiotic control and public health measures should be put in place towards the usage of antibiotics

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Keywords: *Escherichia coli* O157:H7, Antibiotic, susceptibility, Ceftazidime, Multiple antibiotic resistant index.

INTRODUCTION

Urinary tract infections (UTIs) are the most common human infectious disease affecting the bladder, kidneys, ureters and urethra (Tarchouna *et al.,* 2013). It is reported that, 150 million people are affected by UTIs worldwide (Sahib *et al.,* 2012; Emody *et al*., 2003). The incidence of UTIs is higher in women, and it has been estimated that 4050% of adult women experience at least one UTI during their life spans (Tarchouna *et al.,* 2013). Urinary tract infection (UTI) can be caused by bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Proteus* species. *Escherichia coli* is the most common Gram-negative bacterium residing in the gastrointestinal tracts of mammals as commensal organisms causing both community as well as hospital-acquired UTI and it is responsible for more than 85% of all UTIs (Oloketuyi and Khan, 2017),

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It is reported that most *E. coli* strains are also non-pathogenic to humans but the detection of *E. coli* in foods intended for human consumption shows poor in hygiene during production, processing or preparation (Atnafie *et al.,* 2017). It is reported that some strains of *E. coli* are directly pathogenic to humans, example is the shiga toxin-producing *Escherichia coli* O157:H7 (STECO157: H7) which can cause severe enteric infections. *Escherichia coli* O157:H7 is an emerging public health concern in most countries of the world. This was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea traced to consumption of hamburgers at common chains of fast food restaurants (Riley *et al*., 1983). After ingestion of *E. coli* O157:H7, the bacteria bind to the intestinal mucosa and begin releasing shiga toxin. The toxin in turn, disrupts protein synthesis in the epithelial cells lining intestinal mucosa, leading to cell death, sloughing of the mucosa and eventually bloody diarrhea *(Ameer et al., 2021). Escherichia coli* O157:H7 is a major causative agent of severe UTI (Navidinia *et al.,* 2012), responsible for up to 70 to 95% of urinary tract infections (Edberg and Trepeta, 1983). Symptoms of STEC O157 infection may include abdominal pain, bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome (HUS) (Zhao *et al.,* 1995). Chronic colonization with the post-symptomatic shedding of *E. coli O157:H7 may continue to occur, leading to a persistent risk of transmission and re-infection (Ameer et al., 2021).* The aim of this study was to study the prevalence of *E. coli O157:H7* in urine samples of apparently healthy students of a tertiary institution.

MATERIALS AND METHODS

Study Area

This study was conducted in University of Benin, Benin City, Edo State. University of Benin was founded in 1970 as an Institute of Technology and was accorded full University status by the Nigeria National Universities Commission" (NUC) on the 1st of July,1971. The university is located in Benin City, south-south of Nigeria on $6^{0}20.022^{1}$ North latitude and $5^{0}36.009^{1}$ East longitude. It is situated approximately 40km North of the Benin river and 320km by road East of Lagos.

Sample Collection

Informed consent was obtained from University of Benin Ethical Committee, Students were counseled about the research work and consented students were given labeled sterile universal bottles to submit their urine sample. The specimen was appropriately labeled, transported to Microbiology laboratory, Department of Microbiology, University of Benin and were analyzed after collection.

Bacterial Enumeration Isolation and Identification

The media used were prepared following manufacturer's specification. Sterilization of glassware and other autoclavable materials was done at 121°C for 15 min. Urine samples were cultured on Eosin Methylene Blue (EMB) agar and Cysteine Lactose Electrolyte Deficient (CLED) agar with Andrade indicator and incubated for 24 hours at 37 °C as described by Cheesbrough (2006). After incubation, total bacterial count was carried out.

Presumptive *E. coli* isolated from both CLED and EMB were sub cultured on Sorbitol MacConkey agar supplemented with cefixime tellurate supplement (Oxoid) for the selective isolation of presumptive pathogenic *E. coli* O157:H7 strains. The addition of cefixime tellurate supplement inhibits non-0157:H7 *E. coli* strains and other sorbitol non fermenting strains. The isolates were further identified using cultural, morphology, Gram staining and biochemical test such as Indole test and Triple Iron Sugar test.

Antibiotics Susceptibility Test

Few colonies of overnight culture plates were re-suspended in sterile normal saline to match the turbidity of 0.5 McFarland standard for sensitivity testing as described by Cheesbrough (2006). The antibiotic susceptibility of the isolates was determined by the kirby-Bauer disc diffusion method on Mueller Hilton agar following the guidelines of the Clinical and Laboratory Standards Institute (CLSL, 2017). The sensitivity of standard inocula of the isolates to the following antibiotics Ciprofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Floxacin (5µg), Augmentin (30µg), Nitrofurantoin (300µg) (Abtek Biologicals, UK) was carried out. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition was measured in millimeters and interpreted using the CLSI (2017) interpretative chart, and results were taken as resistant, intermediate and sensitive. Multidrug resistance was defined as resistance to three or more classes of antibiotics studied (Magiorakos *et al*., 2012). The multiple antibiotic resistance (MAR) index was determined using the method described by Krumperman (1983) using the formula: a/b, with "a" being the number of antibiotics to which an organism is resistant to and "b" being the total number of antibiotics tested

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RESULTS

Table 1: Percentage occurrence of bacteria isolated from urinary tract of apparently healthy male and female students.

Bacteria Isolated	Male (%)	Female (%)	Total
E. coli	28 (9.2%)	77 (25.3%)	105 (34.5%)
S. aureus	33 (10.9%)	62 (20.4%)	95 (31.3%)
P. mirabilis	14 (4.5%)	23 (7.6%)	37 (12.2%)
Klebsiella spp	48 (15.8%)	19 (6.3%)	67 (22.0%)
Total	123 (40.5%)	181 (59.5%)	304 (100%)

Table 2: Prevalence of *E. coli* and *E. coli* 0157: H7 from Urine samples of Students

Number Examined	Number of E. coli Isolated			Number of *E. coli* 0157: H7 Isolated		
	Males	Females	Total	Males	Females	Total
150	28 (9.2%)	77 (25.3%)	105 (34.5%)	7 (4.67%)	10 (6.67%)	17 (11.33%)

Table 3: Antibiotic Susceptibility Profile of *E. coli* 0157: H7 isolated from urine samples

Isolate Code	Antibiotics							
	CPR	CAZ	CRX	GEN	CXM	OFL	AUG	NIT
***E*1**	R	R	R	S	R	S	R	S
***E*2**	R	R	R	R	R	S	R	S
***E*3**	R	R	R	S	R	S	S	S
***E*4**	R	R	S	S	S	S	S	S
***E*5**	R	R	S	S	S	S	S	S
***E*6**	R	R	S	R	S	S	R	S
***E*7**	R	R	S	S	S	S	R	S
***E*8**	R	R	R	R	S	S	R	S
***E*9**	R	R	S	S	S	S	S	S
***E*10**	R	R	S	R	S	S	R	S
***E*11**	R	R	S	S	R	S	R	S
***E*12**	R	R	R	R	S	S	R	S
***E*13**	R	R	S	S	S	S	R	S
***E*14**	R	R	S	S	S	S	R	S
***E*15**	R	R	S	R	S	S	S	S
***E*16**	R	R	S	S	S	S	S	S
***E*17**	R	R	S	S	S	S	S	S

KEY: *CPR-Ciprofloxacin (5µg), CAZ-Ceftazidime (30µg), CRX-Cefuroxime (30µg), GEN-Gentamicin (10µg), CXM-Cefixime (5µg), OFL-Ofloxacin (5µg), AUG-Augmentin (30µg), NIT-Nitrofurantoin (300µg).*

Table 4: The antibiotic Resistant Profile and the MAR index of *E. coli* 0157: H7 isolated

Isolate Code	Antibiotics Resistance profile	No of Resistance	MAR Index
*E*1	CPR, CAZ, CRX, CXM, AUG	5	0.6
*E*2	CPR, CAZ, CRX, GEN, CXM, AUG	6	0.8
*E*3	CPR, CAZ, CRX, CRX, CXM	5	0.6
*E*4	CPR, CAZ,	2	0.3
*E*5	CPR, CAZ	2	0.3
*E*6	CPR, CAZ, GEN, AUG	4	0.5
*E*7	CPR, CAZ, AUG	3	0.4
*E*8	CPR, CAZ, CRX, GEN, AUG	5	0.6
*E*9	CPR, CAZ,	2	0.3
*E*10	CPR, CAZ, GEN, AUG	4	0.5
*E*11	CPR, CAZ, CXM, AUG	4	0.5
*E*12	CPR, CAZ, CRX, GEN, AUG	5	0.6
*E*13	CPR, CAZ, AUG	3	0.4
*E*14	CPR, CAZ, AUG	3	0.4
*E*15	CPR, CAZ, GEN	3	0.4
*E*16	CPR, CAZ,	2	0.3
*E*17	CPR, CAZ,	2	0.2

KEY: CPR-Ciprofloxacin (5µg), CAZ-Ceftazidime (30µg), CRX-Cefuroxime (30µg), GEN-Gentamicin (10µg), CXM-Cefixime (5µg), OFL-Ofloxacin (5µg), AUG-Augmentin (30µg), NIT-Nitrofurantoin (300µg).

The percentage occurrence of bacteria isolated from apparently healthy male and female students is shown on Table 1. The organisms isolated were; *Staphylococcus aureus*, *Klebsiella* spp, *Proteus mirablis* and *Escherichia coli.* The total prevalence of *E. coli* and *E. coli* 0157: H7 from Urine samples of the study population is presented on Table 2. Whereby out 150 urine sample, 34.5% of *E. coli* and 11.33% of *E. coli* 0157: H7 were isolated. The antibiotic Susceptibility Profile of *E. coli* 0157: H7 isolated from urine samples is shown on Table 3. All the seventeen (17) were resistant to both Ciprofloxacin (5 µg) and Ceftazidime (300 µg). The isolates were found to be 100% susceptible to Ofloxacin (5 µg) and Nitrofurantoin (300 µg). Table 4 is the antibiotic resistant profile and the MAR index of *E. coli* 0157: H7 isolated from the urine samples. It was observed that there was a high multiple drug resistant of *E. coli* 0157: H7 in the study population with MAR > 0.2

DISCUSSION

Urinary tract infection (UTI) remains the most common infectious diseases in both community and hospital acquired settings (Bielaszewska, *et al*., 2005; Bielaszewska, *et al*., 2006). The bacteria isolated were *S. aureus, E. coli, Klebsiella* spp and *P. mirabilis* among the study population is in agreement with the work of Frank-Peterside *et al*. (2006) among students of the University of Port-Harcourt, Ngwai *et al*. (2012) among university students Keffi, Ayoade *et al*. (2013) among University students from redemption campus Ogun State and Gebremariam *et al*. (2019) among students of Mekelle University, northern Ethiopia who also isolated similar organisms. The presence of these organisms could be due to the ascending infection from genital to the urinary tract, climatic and geographic variation and also low sanitary materials in the university such as access of good water supply, low socioeconomic status as reported in Iran by Khoshbakht *et al.* (2013). This implies that these apparently healthy students who are carriers of UTI causing organisms and are likely to come down with UTI.

Female participants recorded the highest percentage of *E. coli* (51.3%) than their male (18.7%) counterparts, which makes the females at high risk of UTI (Forman *et al*., 1997; Wei and Piotr, 2016). This high prevalence of UTI among female participants may be due to females having shorter and wider urethra which is proximate to the anus, having warm and moist urethra which could be supportive for the optimal growth of bacteria compared to males and lack of prostatic fluid which acts as an antimicrobial agent (Melaku *et al*., 2012). Behavioral factors such as the mechanical introduction of pathogens into the bladder and trauma effect during sexual intercourse could also be a reason for this high prevalence of UTI among female individuals (Kurt, 2000). Also, genetic factors, such as blood group secretor status,increasethe likelihood of women contracting UTI organisms (Harrington and Hooton, 2000; Scholes *et al*., 2000).

In this study 11.33% of *E. coli* 0157: H7 was recorded, this is in line with the work of Faten *et al*. (2013) who reported a prevalence rate of 1.5% of *E. coli* O157:H7 from urine samples of children, also, with the result of Navidinia, *et al.* (2012) who reported 2.3 % of *E. coli* isolated from children's urine with UTI. It is reported that *E. coli* O157: H7 infection may involve asymptomatic carriage or uncomplicated diarrhea, but other outcomes include hemorrhagic colitis and hemolyticuremic syndrome (HUS) and infection poses considerable challenges, both clinically and for disease control (Freidrich *et al.,* 2007). Human infections of *E. Coli* O157:H7 have mostly been recognized to be originated from animal source foods (Jo *et al.,* 2004).

In this study, all the 17 isolates of *E. coli* O157:H7 were found to be entirely resistant to ciprofloxacin and Ceftazidime. This might be due to inappropriate or excessive use of antibiotics for therapeutic and prophylactic purpose. The resistance of microorganisms to antibiotics is on the increase and regions of the world recording resistance to different antibiotics, for instance, a study conducted in Saudi Arabia revealed that there was resistantance to nalidixic acid (30µg), cefotaxime (30 µg), and gentamycin (10 µg) (Naser and Wabel, 2007). This variation probably attributed to the expression of resistant genes code by the pathogen which is associated with emerging and re-emerging aspects of the isolates with regards to different agro ecology (Reuben and Owuna, 2013). Increased resistance of bacteria to these antibacterial agents has continued to pose a global threat (Eze *et al*., 2018)

High prevalence of multidrug resistant *E. coli* O157:H7 was reported in this study. All the isolates were resistant to at least 3 classes of antibiotics. Multiple antibiotic resistance (MAR) index is a measure of extent of resistance to antibacterial agents and gives indirect suggestion of the level of risk associated with the probable source of organisms. MAR index of >0.2 indicates isolates are of high risk. The MAR index recorded for all isolates in this study is a pointer to the level of abuse and over use of antibiotics in Nigeria.

CONCLUSION

This study recorded the occurrence of *E. coli* 0157: H7 in the study population. The high resistance to most of the commonly used antibiotics emphasizes the role of antibiotic resistance genes in circulation. Exhibition of multiple antibiotic resistance should be a public health concern since these organisms can find their way to the food chain. Therefore, the implementation of antibiotic stewardship programs is crucial to minimize the chance of selecting for resistant resistance.

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