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Prevalence of Multidrug Resistant Escherichia Coli In Suspected Cases of Urinary Tract Infection Among Patients Attending Ahmadu Bello University Medical Center, Zaria

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Abstract
Urinary tract infections (UTIs) are a major health problem, the second most prevalent human bacterial infection after respiratory tract infection. Patient with urinary tract infection is a potential source of multi drug resistant (MDR) *Escherichia coli* (*E. coli*) with the potentials to spread antimicrobial resistant genes to other bacteria in the environment and other human populations. The aim of the study was to isolate and determine the prevalence of MDR *Escherichia coli* from patients suspected with urinary tract infection attending Ahmadu Bello University Medical Center, Zaria. A total of 95 urine samples were collected and processed according to standard microbiological methods for the isolation and identification of *E. coli*. Antibacterial susceptibility pattern of the isolates was determined using Kirby-Bauer's disk diffusion technique as well as MAR indices. Out of the 95 urine samples collected 32 were from males and 63 were from females, whose ages were between 5 and 74 years. The results revealed that 35 (36.8%) out of the 95 samples collected were positive for *E. coli* with high prevalence among the female patients 23(24.2%) compared to the male patients 12(12.4%). High prevalence of *E. coli* was also reported among the patients within the age ranges of 15-24 and 25-34 years. The *Escherichia coli* isolates demonstrated high resistance to sparfloxacin (91.4%), followed by cotrimoxazole and amoxicillin (82.9%). Additionally, 30 (85.7%) of the isolates exhibited multi drug resistance and 94.3% (n = 33/35) had a MAR index above 0.2. The study demonstrated that some of the *E. coli* isolates in the study are from high-risk contaminated sources where there may be high frequency of antibiotic usage. Therefore, the study indicated the need for Physicians to prescribed antibiotics to patients following standard antibacterial susceptibility testing.
Keywords: Prevalence, *Escherichia coli*, Multi drug resistant, Urinary tract infection

INTRODUCTION

Urinary tract infections (UTIs) are usually caused by bacteria, although they may also be caused by fungi and certain viruses. Gram-negative bacteria from the *Enterobacteriaceae* family, including *Escherichia coli* are among the bacteria. Most of those involved are *E. coli, Klebsiella, Enterobacter, Proteus bacteria*, among others (Foxman and Brown, 2003). However, particularly among young women, some Gram-positive bacteria, mainly *Staphylococcus aureus, Staphylococcus saprophyticus* and *Streptococcus agalactiae*, also play a role. In all patient classes, *Escherichia coli* is the dominant causative agent that triggers 80-90% of all urinary tract infections (Foxman *et al.*, 2000).

Escherichia coli is a natural component of the human and animal intestinal microbiota (Ruiz *et al.*, 2002; Zhang and Foxman, 2003). The distinctive *E. coli* strains that cause most UTIs have been identified uropathogenic *E. coli* (UPEC) (Johnson *et al.*, 2005). They have numerous virulence-related factors (VFs) that assist them in binding, invading, and harming the host, including adhesins, toxins, siderophores, polysaccharide defensive coatings, invasins, and proteins associated with serum resistance (Johnson *et al.*, 2005).

The use of antimicrobials among patients with suspected cases of urinary tract infection has led to the selection of resistant organisms, resulting in dissemination of drug-resistant bacteria (Wenzel *et al.*, 2008; Szmolka and Nagy, 2013). The development of multidrug resistance (MDR; resistance to ≥ 2 antimicrobials) to major classes of antimicrobials within a short period of time after approval for medical use is cause for concern, especially due to the time required to produce new antimicrobial agents (Walsh 2003; Frye and Jackson 2013). Urinary tract infections due to multi drug resistant (MDR) *E. coli* increases the cost of treatment, morbidity and mortality especially in developing countries (Williams *et al.*, 2008; Al-jiffri *et al.*, 2011).

Many of the resistances in MDR *E. coli* are located on plasmids, which increases the possibility of clonal dissemination of these resistance classes in the community (Williamson *et al.*, 2013; Chen *et al.*, 2014). Spread of MDR *E. coli* globally can also be attributed to clones, such as *E. coli* sequence type 131 (ST131), known for its resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole, as well as its virulence and propensity to exchange genetic material, characteristics which further complicate therapy. A number of reports exist on the emergence of this clonal group in *E. coli* from the community and hospital infections in developed and developing countries (Lau *et al.*, 2008; Rogers *et al.*, 2011; Petty *et al.*, 2014). In Nigeria, the emergence of *E. coli* ST131 and ST617 among clinical isolates of *E. coli* was reported in 2012 (Aibinu *et al.*, 2012). However, there is a paucity of data from community infections in Nigeria on the MDR profile of *E. coli* and their genetic lineages or reports on the major clonal complex circulating in the community. The potential spread of MDR *E. coli* is significant, especially for developing countries, which may have low financial resources for healthcare systems and poor infection control management (Adenipekun *et al.*, 2016).

To provide more information on the extent of MDR *E. coli*, the prevalence and antimicrobial resistance of *E. coli* among patients was determined. *E. coli* isolates in this study were tested against a wide range of broad-spectrum antimicrobials. In addition, data on the available antimicrobials that these bacterial organisms are already resistant to is required for proper diagnosis and treatment of bacterial infections in humans.

Experts recommend that the option of antibiotics should be based on the findings of a urine culture and susceptibility test in patients with suspected complicated UTI (Gupta *et al.*, 2001; Scotish Intercollegiate Guidelines Network, 2015). This study aimed at determining prevalence of multidrug resistant *Escherichia coli* from urine of suspected urinary tract infection patients attending Ahmadu Bello University Medical Center, Zaria, Nigeria, in order to generate data that will support clinical decision-making as well as public health and safety.

MATERIALS AND METHODS

Samples collection

A total of ninety-five (95) urine samples were collected in sterilized universal containers following the approval from Ahmadu Bello University Medical Center, Zaria from April, 2019 to August, 2019. The study participants included 32 males and 63 female patients between the ages of 5 and 74 years with suspected cases of UTI. The early morning mid-stream urine samples collected aseptically in a wide mouthed container with screw cap tops, were properly labelled indicating the patients' age, sex, date, and time of collection. Then, the collected samples were transported in ice packs to the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria within 4-6hours of collection, for analysis.

Sample processing

The urine samples were cultured on MacConkey agar aseptically using a wire loop. The plates were incubated aerobically at 37° C for 24hours. After incubation, the cultures were examined for significant bacterial growth based on colonial morphology. The colonies appearing pink suspected to be *Escherichia coli* were picked and sub-cultured onto nutrient agar slants for isolation of pure colonies and then incubated at 37° C for 24 hours (Collee *et al.*, 1996; Cheesbrough, 2006).

Identification of isolates

The isolates were identified based on colonial morphology, Gram staining reaction, confirmatory test and appropriate biochemical tests such as sugar fermentation test using Triple Sugar Iron Agar, oxidase test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test and ONPG (Ortho-nitrophenyl Beta-D-galactopyranoside) test (Collee *et al.*, 1996; Cheesbrough, 2006).

Antibiotics susceptibility testing

All isolates were tested for antibiotics susceptibility first by standardizing the inocula and then inoculating onto Mueller-Hinton agar by the Kirby-Bauer disc diffusion method (Bauer, 1966) recommended by the Clinical and Laboratory Standards Institute (CLSI, 2015). Antibiotics Disks containing Cotrimoxazole (30µg), Gentamicin (30µg), Ciprofloxacin (30µg), Chloramphenicol (30µg), Augmentin (10µg), Perfloxacin (30µg), Sparfloxacin (30µg), Streptomycin (30µg), Amoxicillin (30µg) and Ofloxacin (10µg) were used against the isolates. *Escherichia coli*, ATCC25922, was used as the quality control strain (Cheesebrough, 2006). Susceptibility test results were interpreted according to the criteria established by the (CLSI, 2015). The results were reported as sensitive, intermediate and resistant according to Clinical Laboratory Standards Institute (CLSI, 2015) guide lines. An isolate was defined as being multi-drug resistant, when it was found to be resistant to three or more of the antibacterial agents tested and based on the antimicrobial categories (Margiorakos *et al.*, 2012).

Determination of multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance (MAR) index for each isolate was determined using the formula MAR = a/b, where '*a*' is the number of antibiotics to which the test isolate was resistant to and '*b*' is the total number of antibiotics to which the test isolates were subjected (Sandhu *etal*., 2016)

Statistical analysis

Data generated were analyzed using Chi-square analysis (SAS version 9.1.3). P -value less than or equals to 0.05 ($p \leq 0.05$) were considered significant.

RESULTS

The result indicated that 60 (63.2%) of the urine samples were negative and 35 (36.8%) were positive for *E. coli*. Moreover, high prevalence cases were identified among patients between 25-34 and 15-24 age groups with (46.2%) and (40%) prevalence respectively (Table 1). The differences are not statistically significance (P=0.497).

Table 1: Prevalence of *Escherichia coli* based on age group of patients

Age(Years)	No. Examined	No. Positive	Prevalence (%)
5-14	9	3	33.3
15-24	25	10	40
25-34	13	6	46.2
35-44	33	12	36.4
45-54	10	3	30
55-64	0	0	0
65-74	5	1	20
Total	**95**	**35**	**36.8**

P = 0.4971

The prevalence of *Escherichia coli* according to gender of the patients reveals that 12 (37.5%) males were positive while 23 (36.5%) were female patients (Table 2). The highest prevalence of *E. coli* based on gender was observed in female patients. However, based on statistical analysis it was observed to be not significant (P = 0.9245).

Table 2: Prevalence of *Escherichia coli* based on gender of patients

Gender	No. Examined	No. Positive	Prevalence (%)
Male	32	12	37.5
Female	63	23	36.5
Total	**95**	**35**	**36.8**

P = 0.9245

The drug susceptibility pattern of the *Escherichia coli* isolates against the 10 different antibiotics was shown in Table 3. The findings of the study showed that *E. coli* was highly susceptible to augmentin (71.1%) followed by gentamicin and chloramphenicol (68.6%). However, the *Escherichia coli* isolates demonstrated high resistance to sparfloxacin (91.4%), followed by cotrimoxazole and amoxicillin (82.9%) and ciprofloxacin (71.4%).

Table 3: Antibiotics susceptibility pattern of *Escherichia coli* isolates from urine samples

Antibiotics (disk conc. in µg)	Susceptibility of *E. coli* (n = 35)	
	Sensitive (%)	Resistant (%)
SXT (30)	6 (17.1)	29 (82.9)
CH (30)	24 (68.6)	11 (31.4)
SP (10)	3 (8.6)	32 (91.4)
CPX (30)	10 (28.6)	25 (71.4)
AM (30)	6 (17.1)	29 (82.9)
AU (10)	25 (71.1)	10 (28.6)
GN (30)	24 (68.6)	11 (31.4)
PEF (30)	8 (22.9)	27 (77.1)
OFX (10)	12 (34.3)	23 (65.7)
S (30)	21 (60)	14 (40)

Key: n = number of isolates, SXT = Cotrimoxazole, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, AM = Amoxacillin, AU = Augmentin, GN = Gentamicin, PEF = Perfloxacin, OFX = Ofloxacin, S =Streptomycin.

Figure 1 describes the prevalence of MDR *E. coli*, a total of 30 (85.7%) isolates were found to be MDR *E. coli*. While Table 4 present resistance patterns and multiple antibiotic resistance index. This study observed that 94.3% (n = 33/35) of the isolates had multiple antibiotic resistance (MAR) index greater than 0.2 while 5.7% (n = 2/35) isolates had MAR index of 0.2.

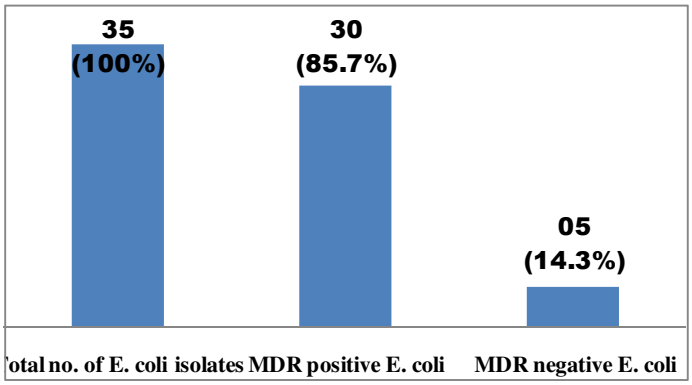

Figure 1: Prevalence of Multi drug resistant (MDR) *Escherichia coli* from urine samples of suspected urinary tract infection patients attending Ahmadu Bello University Medical Center, Zaria.

Table 4: Antibiotic resistance pattern and MAR Index in *Escherichia coli* isolates

S/N	Isolate Code	Resistance pattern	MAR Index
1	AMC3	SXT, SP, AM, AU,PEF, OFX	0.6
2	AMC 5	CH, CPX,AM,PEF,S	0.5
3	AMC 6	SXT,SP,AM,PEF,OFX	0.5
4	AMC 8	SXT, CH,SP,AM,AU,PEF,OFX	0.7
5	AMC 11	STX,SP,CPX,AM,PEF,S	0.6
6	AMC 21	SXT,CH,SP,CPX,AM,AU,GN,PEF,OFX	0.9
7	AMC 25	SP, CPX, S	0.3
8	AMC 27	SXT,CH,SP,CPX,AM,PE,OFX,S	0.8
9	AMC 28	SXT,SP,CPX,AU, PEF,OFX	0.6
10	AMC 32	SXT,CH,CPX,AM,PEF,S	0.6
11	AMC 38	SXT,SP,CPX,AM,GN,OFX	0.6
12	AMC 40	SXT,CH,SP,CPX,AM,AU,GN,PEF,OFX	0.9
13	AMC 41	SXT,SP,CPX,	0.3
14	AMC 42	SXT,CH,SPAM,AU,GN,OFX	0.7
15	AMC 44	SP,CPX,AM,PEF,OFX,S	0.6
16	AMC 47	SXT,CH,SP,CPX,AM,PEF	0.6
17	AMC 49	SXT,SP,AM,AU,GEN,OFX	0.6
18	AMC 50	SXT,CH,SP,CPX,AM,PEF,S	0.7
19	AMC 51	SXT,SP,CPX,AM,PEF,OFX	0.6
20	AMC 53	CH,SP,AM,AU,GN,OFX,S	0.7
21	AMC 58	SXT,SP,CPX,AM,PEF,OFX	0.6
22	AMC 61	SXT,SP,CPX,AM,AU,GN,PEF,OFX,S	0.9
23	AMC 63	SP,AM	0.2
24	AMC 64	SXT,CH,SP,AM,GN,OXF,S	0.7
25	AMC 66	SXT,SP,CPX,AM,PEF	0.5
26	AMC 69	SXT,SP,CPX,GN,PEF,OFX,S	0.7
27	AMC 70	CPX,AM	0.2
28	AMC 71	SXT,SP,CPX,AU,GN,OFX,S	0.7
29	AMC 74	SXT,SP,CPX,AM,PEF,OFX	0.6
30	AMC 75	SXT,SP,CPX,AM,GN,OFX,S	0.7
31	AMC 79	SXT,SP,CPX,AM,PEF,OFX	0.6
32	AMC 83	SXT,SP,CPX,AM,GN,PEF,OFX,S	0.8
33	AMC 86	SXT,SP,AM,GN,PEF	0.5
34	AMC 90	SXT,SP,PEF	0.3
35	AMC 92	SXT,SP,CPX,AM,GN,PEF,OFX	0.7

Key: AMC = Ahmadu Bello University Medical Centre, AMR= Multiple Antibiotic Resistant, SXT = Cotrimoxazole, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, AM = Amoxacillin, AU = Augmentin, GN = Gentamicin, PEF = Perfloxacin, OFX = Ofloxacin, S =Streptomycin.

DISCUSSION

The findings of this study reveal that the prevalence of *E. coli* among the studied subjects was 36.8%. This implies that a significant number of patients suspected with UTI may harbour *E. coli*. This observation may not be unconnected with the fact that *E. coli* is a natural component of the human and animal intestinal microbiota as documented by Ruiz *et al*. (2002); Zhang and Foxman (2003), however its presence in the urine samples of the studied subject who were patients suspected with UTIs is a source of concern.

Compared to the findings of this study, Aboderin *et al*. (2009) reported lower prevalence of *E. coli* of 25.6%, Karuiki *et al*. (2007), reported 36% in Nairobi, Kenya. Sanitary conditions as well as hygiene practices among patients may have accounted for the disparity in the prevalence observed.

In line with the observation of studies by Eghieye *et al*. (2018); Farouk *et al*. (2019), this study also revealed that the prevalence of *Escherichia coli* was higher among those aged 15-24 years and 25-34 years. The high prevalence of *E. coli* in these age groups may be related to the fact that patients in this age group are sexually active and possibly to have been infected with uropathogenic *E. coli*.

In terms of gender, the prevalence of *E. coli* was found to be higher in females than it was in males. This is similar with the findings of Eghieye *et al,* 2018 and Farouk *et al.*, 2019. The near proximity of the urogenital tract to the anus in women may account for the higher percentage observed among women, makes it possible for *Escherichia coli*, typically located in the gastrointestinal system, to be conveyed to the female genital tract (Ovalle and Levancini, 2001). The lower prevalence *E. coli* detected in men may be due to the greater length of the male urethra and the antibacterial activity of male prostatic fluid (Gupta and Stamm, 2005).

The study demonstrated that almost all the *E. coli* isolates obtained in the study were resistant to two or more antibiotics with 85.7% of them identified as MDR. This observation demonstrated that *E. coli* isolated in the study were highly resistant and is a source of public health concern.

This high degree of resistance found in *E. coli* could be attributed to the indiscriminate use of antibiotics, over-prescription, poor quality and cheaper medicines that are being marketed and delivered to patients that do not have urinary tract infection (Batabyal and Himanshu, 2018). In a study, Szmolka and Nagy (2013) noted that drug resistant commensal *E. coli* remains a reservoir of drug resistance and may also be responsible for the resistance pattern observed in their study.

Similar to the reports of this study, other studies on the prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria, reported 81.3% prevalence of MDR *E. coli* (Kamweli *et al.*, 2019). Another survey of households and chicken farms in the Mekong Delta in Vietnam reported a high prevalence of multidrug resistant *E. coli* (81.3%) (Campbell *et al.*, 2015). However, some studies revealed lower prevalence of MDR *E. coli*. For instance, a study done in Korea among poultry farm workers reported that 43% of *E. coli* isolated from the workers showed resistance to four or more antimicrobials used in poultry production (Cho *et al.*, 2012). Similarly, another study in the Netherlands reported that 27% of *E. coli* isolated from broiler and layer chicken farmers showed resistance to more than three antimicrobials (Bogaard, 2009).

It has been documented that multiple antibiotic resistance is mostly observed by the action of multidrug efflux pumps, each of which can pump out more than one drug type (Nikaido *et al.*, 2009).

The findings of this study showed that 94.3% of *E. coli* isolates had MAR index greater than 0.2. Studies have shown that MAR indices greater than 0.2 implies isolates from high-risk contaminated sources with frequency of antibiotic use (Thenmozhi *et al.*, 2014). This may be correlated with the indiscriminate use of antimicrobials among patients with suspected UTI for therapy purposes.

The findings of this study also demonstrates that the *E. coli* isolates obtained from the urine of patients with suspected UTI showed high resistance rates against sparfloxacin (91.43%), cotrimoxazole (82.86%) and amoxicillin (82.86%), ciprofloxacin (71.43%) and ofloxacin (65.71%). However, few of the isolates were found to be susceptible to other antibiotics, particularly streptomycin, augmentin, gentamicin and chloramphenicol. A similar study in Nigeria reported high resistance rates against amoxicillin, cotrimoxazole and ciprofloxacin (Sabir *et al.*, 2014; Namrathan, 2015; Farouk *et al.*, 2019). A possible explanation for MDR *E. coli* observed among the patients may be due to the widespread use and easy availability of these drugs, which has led to the high resistance pattern of the isolates.

CONCLUSION

The study has observed a high prevalence rate of MDR *E. coli* (85.7%) among patients with suspected cases of urinary tract infection attending Ahmadu Bello University Medical Center, Zaria. The *Escherichia coli* isolates demonstrated high resistance to sparfloxacin (91.4%), followed by cotrimoxazole and amoxicillin (82.9%). The prevalence of MDR *Escherichia coli* isolates with MAR indices greater than 0.2 is a threat to the ongoing campaign against antibiotic misuse. Therefore, strict measures in the administration of antibiotics should be adhered to by Clinicians in the treatment of urinary tract infection and drugs should only be prescribed following antibiotic sensitivity testing of the isolates.

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