



Incidence and Biogram of *Escherichia coli* 0157:H7 Serotype among Cases of Urinary Tract Infections in a Secondary Health Facility in Nigeria

F. A. Olajubu¹, I. B. Olushola², A.A. Mekoma³, , A. F. Ogunmodede⁴

^{1,2,3}Department of Microbiology, Adegunle Ajasin University, Akungba-Akoko, Nigeria

⁴Paediatrics Department, State Specialist Hospital, Ikare-Akoko, Nigeria

*Corresponding Author: festus.olajubu@aaau.edu.ng

Abstract

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Escherichia coli is capable of causing widespread infections such as diarrhea and urinary tract infection thereby becoming a public health issue. This study investigates the incidence of urinary tract infections caused by *Escherichia coli* serotype 0157:H7 among patients attending the State Specialist Hospital Ikare-Akoko, Ondo State. One hundred and twenty (120) clean catch mid-stream urine samples collected from forty (40) males and eighty (80) female patients, were cultured on Cystin Lactose Electrolyte Deficiency (CLED) and Eosin Methylene Blue (EMB) media. Gram's staining and biochemical tests were done on the isolates. The *E. coli* isolates were screened for serotype 0157:H7. Antibiotic susceptibility test using disk diffusion technique was carried out on the isolates. The susceptibility of the isolates to ethanolic extracts of *Piptadeniastrum africanum* was equally assessed. The incidence rate of urinary tract infection among the studied population is 58.3%. The most frequently isolated bacterium is *Escherichia coli* with a total of 32 (45.7%) isolates out of which 11 (34.4%) were of the serotype 0157:H7. Eighty-six percent (86%) of the isolates were sensitive to Nitrofurantoin and Ciprofloxacin, while Ampicillin, Tetracycline, Cephalexin, and Cotrimoxazole showed resistance. 70% of the isolates were sensitive to the extracts of *Piptadeniastrum africanum*. This study revealed that *E. coli* including serotype 0157:H7 was the predominant organism responsible for UTI among the studied population.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections found in developing countries like Nigeria where proper sanitization is inadequate. Though, many species of *E. coli* are harmless especially when found in the colon, others cause diarrhea, septicemia, UTI and pneumonia or generally respiratory illnesses (Perez *et al.*, 2004). The common etiologic agents of UTI include enterobacteriaceae like *E. coli* and *Klebsiella spp.*, as well as Gram positive organisms like Staphylococci and enterococci (Farshad *et al.*, 2010; Litwin, *et al.*, 2005). Studies carried out in Enugu, Yola, Zaria and Ife show that these are the same etiological agents

isolated in Nigeria. (Mbata, 2007). It has been reported that more than 150 million people are affected by UTI globally (Smith *et al.*, 2003; Stamm and Norrby, 2001) and it has also been estimated that about 30,000 UTI patients are treated in clinical wards from 6 million patients that are visited for UTI globally per year, particularly infants, pregnant women, elderly of both sexes as well as patients with spinal cord injuries, indwelling catheters, diabetes, multiple sclerosis, acquired immune deficiency and underlying urological abnormalities (Maji *et al.*, 2013; Svanberg-Eden and Cindo, 2009).

Escherichia coli is also one of the most important causes of community-acquired and nosocomial

infections, this organism is therefore of clinical importance from various clinical specimen particularly strains of enterohemorrhagic *Escherichia coli* (EHEC) serotype 0157:H7 which are responsible for outbreaks of hemolytic uremic syndrome (HUS) worldwide. (Umolu *et al.*, 2006). Knowledge on the antimicrobial pattern in *E. coli*, the predominant pathogen associated with urinary tract infection is important as a guide in selecting empirical antimicrobial therapy (Aghazadeh *et al.*, 2015). The use of plants for medicines is an ancient practice common to all societies especially the African society. *Piptadeniastrum africanum* is commonly used in traditional medicine, mostly the bark, sometimes also roots and leaves. Bark decoctions are used internally to treat urinary tract infections, stomach-ache, dysmenorrhoea and male impotence, and as an antidote; externally, they are applied to treat toothache, pneumonia, oedema, skin complaints and rheumatism, to expel worms, to dispel fleas, and as a purgative and abortifacient. (Neuwinger, 2006). This study therefore investigated the involvement of *E. coli* serotype 0157:H7 in UTI cases among the studied population. The susceptibility of these isolates to ethanolic extracts of *P. africanum* were also assessed.

MATERIALS AND METHODS

Specimen collection

One hundred and twenty (120) clean catch mid-stream urine samples were aseptically collected from patients (40 males and 80 females) attending the State Specialist Hospital Ikare-Akoko (SSHI), Ondo State, Nigeria with suspected urinary tract infection (symptomatic), from June 2017 to November 2017 (a six-month study). These clean catch mid stream urine samples were collected into labeled, sterile, wide mouthed, leak proof, plastic universal containers and were then transported to the laboratory and processed within an hour of collection. Fifteen (15) urine samples which were poorly collected and thus heavily contaminated were excluded from the samples treated.

Macroscopic and microscopic examination

Macroscopic examination of collected urine samples was done for color and appearance and were reported accordingly. In microscopic examination, urine samples were centrifuged at 3000 rpm for 5 mins, deposits were prepared and examined under the Microscope.

Urine culturing and bacteria identification

Urine specimens were cultured on Cysteine Lactose Electrolyte Deficient (CLED) and Eosin Methylene Blue (EMB) agar plates by calibrated loop of 1 µL after thoroughly mixing them and incubated at 37°C for 24h. Specimens with more than 10⁵ colony forming unit (CFU) per ml were considered as positive samples. One positive culture only per patient were considered in this study. All isolates were identified culturally, morphologically and biochemically by standard laboratory procedures. Identified isolates were stored in agar slant for use in further studies.

Antibiotic susceptibility test

Antimicrobial susceptibility test was performed according to "Kirby and Bauer" method on Mueller-Hinton agar medium. The antimicrobial agents used for the susceptibility testing of the isolates were Ampicillin (10µg), Gentamicin (10µg), Nitrofurantoin (30µg), Cotrimoxazole (30µg), Ofloxacin (5µg), Ciprofloxacin (5µg), Cephalexin (5µg), Tetracycline (10µg). These are common antibiotics used by the studied community.

Method of extraction:

A 50gm portion of air-dried, powdered leaves of *P. africanum* was soaked in 1 litre of ethanol for 72hrs according to the methods of Rojas *et al* (2006). The mixture was refluxed, agitated at 200rpm for 1hr and filtered using Whatman No1 filter paper. The filtrate was placed in a vacuum oven at 40°C and dried for 3 days to obtain the dry extract. The extract was stored at 4°C until it was needed for use.

Test of antimicrobial efficacy of *P. africanum* extracts on the isolates.

The agar well diffusion method of Perez *et al.*, (2004) was used. The extract was dissolved in Dimethyl sulfoxide (DMSO) and used at a concentration of 100mg/ml. Each well was filled with 0.2ml of the standardized extract after being seeded with the desired organism. The plates were incubated at 37°C for 24hrs. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extracts.

Serotyping of *E. coli* isolates

This test was done to identify the *E. coli* of the serotype O157:H7 using *E. coli* O157 latex test kit (Oxoid).

RESULTS

Out of 120 clean catch mid-stream urine samples that were aseptically collected and analyzed, which include (40 males and 80 female patients), between the ages of 10 years to 75 years, 70 samples had significant growth. 55 (78.6%) females came up with positive cultures while 15 (21.4%) males had significant bacteriuria as shown in Table 1.

Five different microorganisms were identified from the positive cultures. *E. coli* was responsible for 32 (45.7%) of the cases of UTI. This is followed by

Staphylococcus aureus 13 (18.6%), *Klebsiella pneumonia* 10 (14.3%), *Pseudomonas aeruginosa* 9 (12.9%), and *Enterococcus faecalis* 6 (8.5%) as shown in Table 2.

Table 3, shows the antibiotic susceptibility pattern of the *E. coli* isolates, Nitrofurantoin was the most active of all the antibiotic used, followed by Ciprofloxacin, Gentamycin and Ofloxacin while Ampicillin, Tetracycline, Cephalexin, and Cotrimoxazole showed resistance. The antimicrobial patterns of *P. africanum* on the isolates is shown in Table 4. Leaf extracts shows to be more effective than the stem extracts.

The *E. coli* O157 typing test result is shown on Figure 1. Eleven (34.4%) of the *E. coli* isolates were positive while 29 (66.6%) were negative

Table 1: Gender Distribution of Positive Urines Samples.

S/No	Gender	No of samples (%)	No of positive samples(%)
1	Male	40 (33.3)	15(21.4)
2	Female	80(66.7)	55(78.6)
Total		120	70

Table 2: Frequency of occurrence of the bacteria isolated from the urine.

Bacterial Isolate	Male (%)	Female(%)	Total(%)
<i>Escherichia coli</i>	6 (8.6)	26 (37.1)	32 (45.7)
<i>Staphylococcus aureus</i>	3(4.3)	10 (14.3)	13 (18.6)
<i>Pseudomonas aeruginosa</i>	2 (2.9)	7 (10)	9 (12.9)
<i>Klebsiella pneumonia</i>	2 (2.9)	8 (11.4)	10 (14.3)
<i>Enterococcus faecalis</i>	2 (2.9)	4 (5.7)	6 (8.5)
Total	15	55	70

Table 3: Antibiotic susceptibility pattern of the *E. coli* isolates.

Antibiotic	Sensitive (%)	Intermediate(%)	Resistant(%)
Nitrofurantoin	30 (93.7)	2(6.3)	-
Ofloxacin	25 (78.1)	7(21.9)	-
Gentamicin	-	26(81.3)	6(18.7)
Cotrimazole	-	3 (9.4)	29(90.6)
Ciprofloxacin	28 (87.5)	4 (12.5)	-
Cephalexin	-	3(9.4)	29 (90.6)
Tetracycline	-	17(53.1)	15(46.9)
Ampicillin	-	-	32(100)

Table 4: Antibacterial activity of *Piptadeniastrum africanum* on the isolates.

Extract Conc	Plant Parts	
	Stem No Sens. (Res)	Leaf No Sens. (Res)
100mg	26(6)	30(2)
50mg	0(32)	27(5)
25mg	0(32)	12(20)

Key: Figures in bracket are resistant.

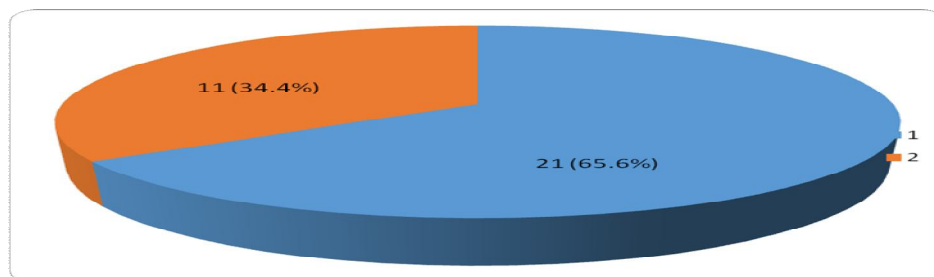


Figure 1. Frequency of 0157:H7 and non 0157:H7 serotypes of *E. coli* from the urine samples

- 1 - Non 0157:H7
- 2 - 0157:H7

Table 5: Antimicrobial susceptibility and efficacy assessment of *P. africanum* on 0157:H7 serotypes

Antibiotics/Extracts	No Sensitive	% Sensitive
Nitrofurantoin	7	63.6
Ofloxacin	4	57.1
Gentamycin	-	0
Cotrimoxazole	-	0
Ciprofloxacin	4	57.1
Cephalexin	-	0
Tetracycline	-	0
Ampicillin	-	0
Stem bark (100mg)	9	81.8
Leaf (100mg)	9	81.8

DISCUSSION

The overall occurrence of urinary tract infection in this study is 58.4%. Various incidence rates have been reported from as low as 13.1% to as high as 84% (Iregbu and Nwajiobi-Princewill, 2013; Subhashini, 2016). These variations might be as a result of prevailing socio-economic factors and

cultural behaviours of the individual population. Non-availability and accessibility to health information and health facilities might equally be a contributing factor. *E. coli* was responsible for 45.7% of all isolates thereby making them the most isolated. The predominance of *E. coli* in this study is in agreement with various studies earlier reported (Afriyie et al., 2015; Gizachew et al., 2013; Ogbukagu et al., 2016). The cause of high incidence infection with *E. coli* might be due to the fact that this bacteria leave their natural place (micro flora of the intestine) to urinary pathways causing inflammation of urinary tract because they possess numerous virulence factors such as Pili which helps in adhesion to epithelia of urinary system. *E. coli* is also ubiquitous in nature though, primarily of the intestinal tract. It is an environmental contaminant and often contaminates bathing waters or water used for cleaning up after defecation. Previous case of UTI without proper treatment can make an individual become a reservoir for the organism.

In general, girls and women are more vulnerable to UTI than boys and men as demonstrated by observation in this study (Asscher et al., 1998). Many other studies have reported higher infection rates in female than male as observed in this study (Aghazadeh et al., 2015; Subhashini et al., 2016). The higher occurrence rate recorded in females could

be due to varieties of factors, it could be anatomical, where short urethral as well as the closeness of vagina and anus favour contamination of the vagina by intestinal flora in prevailing poor hygiene circumstances, alteration of the in vaginal microflora also play a critical role in encouraging vaginal colonization with coliforms which can lead to Urinary Tract Infection. (Ameil et al., 2003).

In addition, the urine of female has been found to have a more suitable pH and osmolarity for the growth of *E. coli* and other enterics in urine (Perez et al., 2004). Most of the bacteria isolates encountered are normal flora of fecal matters. The spread of normal flora in fecal materials from the anus to the vaginal from where the bladder could be infected as a result of poor anal cleaning could be responsible for the observed results in female's urine samples (Azubike et al., 2004; Perez et al., 2004). This is an indication of poor hygiene practices and poor sanitations in the environment. Therefore, the need for good personal hygiene practices in the society is pertinent.

It is also likely that some of the cause of the infections especially among hospitalized patients would have been acquired from hospital sources. The role of hospital facilities and personnel as well as certain medical practices in introducing urinary tract infection aetiologic agents is well documented (Gadea et al., 2012; Hany, 2008).

The antibiotic susceptibility pattern of the isolates from infected urine samples showed complete resistance to Cotrimoxazole, Tetracycline and Ampicillin. Gentamicin showed very low effectiveness on the isolates. Nitrofurantoin, Ciprofloxacin and Ofloxacin showed excellent antimicrobial activity. This study shows Nitrofurantoin and Ciprofloxacin to be highly sensitive to all urinary isolates of *Escherichia coli*. The consistent and high level susceptibility of *Escherichia coli* to nitrofurantoin may be influenced by nitrofurantoin's broad spectrum activity, limited indication (treatment of acute cystitis), narrow tissue distribution and limited contact with bacteria outside the urinary tract according to James et al.,(2002). Antibacterial resistance may occur due to beta-lactamase production, cell membrane alterations, reducing antibiotic uptake (Gram negative bacteria), or inactivation of the drugs. Increase in antimicrobial resistance may be due to the extensive use and misuse of antimicrobial drugs which have favored the emergence and survival of resistant strains of micro-organisms (Okonofua et al., 1989; James et al.,2002).

The antimicrobial assay of *Piptadeniastrum africanum* on the isolates from infected urine samples shows effectiveness on the isolates, the leaf extracts were more effective than the stem bark of plant extracts. Joy and Adeola(2009), in a similar study attached the efficacy of extracts of *P. africanum* to the presence of components such as vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemisinin and ephedrine among others. In the rising occurrence of multidrug resistance among various bacteria species, extracts of *P. africanum*, may be an alternative medication.

Eleven (34.4%) *E.coli* O157:H7 were isolated from UTI cases in this study even though, the serotype are commonly associated with diarrhea disease. This result is compatible with the study of Navidinia, et al., (2012) who showed that 28.3 % of *E.coli* isolated from patient's urine with UTI were enterohemorrhagic. Shiga-toxin *E. coli* (STEC) has been isolated from urine samples which were of the O157:H7 serotype, however, no toxin production was checked for in the current study (Wilson et al., 200). The presence of *E. coli* O157:H7 serotype in the sampled urines requires urgent control measures (like adequate treatment) to curb its further spread.*E. coli* O157:H7 were reasonably susceptible to the leaves and stem bark extracts of *P. africanum* which might serve as alternative sources of treatment for the local community who were already used to the plant for treatment of UTI and other ailments.

CONCLUSION

Escherichia coli was found to be the most predominant bacterium isolated from urine samples analyzed with higher occurrence rate in females. The isolates were sensitive to Nitrofurantoin, Ciprofloxacin and Ofloxacin which may be useful for the treatment of UTIs empirically among the studied population. The result of antimicrobial susceptibility assay of *P. africanum* against *E. coli* indicated an alternate treatment regimen for those infected. Isolation of O157:H7 serotype of *E. coli* calls for regular surveillance exercise and adequate treatment of patients to curb any further envisaged spread.

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