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Antimicrobial Susceptibility Pattern of *Escherichia Coli* Isolates from Clinical Sources at Tertiary Health Care Setting, Ile Ife, South Western Nigeria

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Abstract

Antimicrobial resistance is majorly an issue of public health concern. The aim of this study is to isolate and identify *Escherichia coli* from samples of stool and urine obtained from the clinical settings at Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, and to determine their antibiotics susceptibility patterns. Nineteen (19) of stools and 22 of urine samples were analysed using standard microbiological and biochemical techniques and 11 pure isolates were obtained comprised of 5 (12,2%) isolates from urine and 6 (14,6%) isolates from stool. Antibiotics susceptibility studies were conducted using Kirby and Bauer disc diffusion method, and the results were determined using the Clinical and Laboratory Standards Institute (CLSI) guides.

The studies showed that all the *E. coli* isolated were 100% resistant to augmentin, gentamycin, streptomycin, tetracyclin and chloramphenicol, and 90.90% resistant to oflaxin, sparfloxacin, and amoxycillin while the isolates were susceptible tociprofloxacin (26.33%), and pefloxacin (45.46%).

Effective hygiene must be encouraged and indiscriminate usage of antibiotic must be avoided in the study area.

Keywords: Antibiotics; Clinical; *Escherichia coli*; Urinary tract infection; Susceptibility pattern

Introduction

Antimicrobial resistance has become an issue of public health concern and is a major factor contributing to mortality and morbidity in settings with limited diagnostic facilities and treatment options. *Escherichia coli* accounts for 17.3% of clinical infections requiring hospitalization and is the second most common source of infection next to *Staphylococcus* aurous among out-patient infections.

Studies on commensal bacteria in developing countries have shown high resistance rates to diverse antimicrobial agents [1]. In developing countries, because of diarrhea associated infection, 2.2 million people die every year and annually, 130-175 million patients suffer uncomplicated Urinary Tract Infection (UTI) worldwide and more than 80% of them are due to *E. coli* [2-4].

Despite concerning trend in antimicrobial resistance among *E. coli* isolates worldwide, a growing armamentarium of antimicrobial agents provides multiple options for treating *E. coli* infections [5]. In general, monotherapy with trimethoprim-sulfamethoxazole, aminoglycosides, cephalosporin or fluoroquinolones is recommended as the treatment of choice for most known infections with *E. coli*, though many broad spectrum agents (such as beta-lactam/beta lactamase inhibitor combinations and the carbapenems) remain highly active.

Pathogenic *E. coli* strains use a multi-step scheme of pathogenesis that is similar to that used by other mucosal pathogens, which consists of colonization of a mucosal site, evasion of host defenses, multiplication and host damage [6,7]. There is an alternate side to *E. coli* afforded through gene gain and loss that enables it to become a highly diverse and adapted pathogen [8]. Pathogenic *E. coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream and central nervous system [6].

In this study we aimed at screening and assessing the antibiotic susceptibility pattern of *E. coli* isolated from clinical

specimens in the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria.

Materials and Methods

Study area

The clinical samples used were obtained from the Microbiology and Parasitology Department of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria in October, 2015.

Sample population

A total of 41 samples comprising 19 stool samples and 22 urine samples were taken from the Microbiology and Parasitology Department, Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. The samples were transferred to the Department of Microbiology Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria for subsequent culturing and biochemical tests [9,10].

Bacterial isolation and identification

Culture plates from Deoxycholate agar (Oxoid, UK), MacConkey agar (Oxoid), nutrient agar, blood agar were used. The swab sticks used for the collection of the samples were streaked directly on the labelled agar plate and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Biochemical test were performed to identify microbes that could not be characterized by morphology. Biochemical tests applied were standard catalase test, citrate utilization, oxidase, Voges- Prokauer, indole production, motility, sucrose, maltose, lactose, nitrate reduction and mannitol [11].

Antibiotic susceptibility

In vitro susceptibility of the isolates against microbial agents was determined by the standard disc diffusion procedure (Bauer et al., 1996). The following antibiotics were used Augmentin (30 µg), Gentamycin (10 µg), Oflaxacin (10 µg), Streptomycin (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (5 µg), Amoxycillin (30 µg), Pefloxacin (30 µg). Identified isolates of *E. coli* were inoculated into nutrient broth at 37°C for 24 hours. The zones of inhibition were measured, recorded and interpreted according to the Clinical Laboratory Standard Institute provided [12].

Results and Discussion

In this study, eleven (11) isolates of *Escherichia coli* were obtained from 41 samples. **Table 1** shows the percentage distribution of *E. coli* in relation to age and sex. Female individuals falling within the age range 21-30, 31-40, 41-50 constituted higher *E. coli* infection rates of 18.18% each. The rest of the individuals were 9.09% each.

Table 1 Distribution of *E. coli* infections in relation to age and sex.

Age range (years)	Isolate code	Total patient samples	Percentage distribution	
			Males	Females
0-10	-	-	-	-
11-20	U3	2	9.09%	9.09%
	U12	-	-	-
	S2	-	-	-
21-30	S10	3	9.09%	18.18%
	S17			
31-40	S11	2	0%	18.18%
	S12	-	-	-
41-50	S4	-	-	-
	U9b	3	9.09%	18.18%
	U17c			
51-60	U7b	1	9.09%	0%
61-70	-	-	-	-
71-80	-	-	-	-
81-90	-	-	-	-
91-100	-	-	-	-

Table 2 shows the distribution of *E. coli* infection in polymicrobial association and variable medical diagnosis. Urinary Tract Infection (27.27%) was predominantly associated with *E. coli* followed by other implicated clinical conditions ranging from Gastroenteritis, RVDX, BAA to CAP which constituted 9.09% each of the mixed infection.

Table 2 Distribution of *E. coli* infection case histories.

Case Histories	Isolate Codes	Total number %
Gastroenteritis	S2	9.09%
Typhoid	S4	9.09%
Medical Test	S10	-
Medical Test	S11	-
Medical Test	S17	36.36%
Medical test	U9b	-
Urinary Tract Infection	U3	-
Urinary Tract Infection	U12	27.27%
Urinary Tract Infection	U17c	-
Retroviral Disease (RVDS)	S12	9.09%
BAA to CAP	U7b	9.09%
Total		100%

Morphological characteristics of colonies were recorded on the media used (MacConkey, Sugars, EMB) for primary

identification of *E. coli*. A total of 11 isolates were retrieved from 4 males and 7 females. All the isolates were Gram's negative showing pinkish colonies on MacConkey agar and greenish metallic sheen on Eosin Methylene Blue agar (**Table 3**). **Table 4** shows the biochemical characteristics of the isolates.

Table 3: Cultural morphology of *E. coli* isolated from clinical sources.

Sources of isolation	Sex	Diagnosis	Isolate code	Gram stain	Growth on Mac	Growth on EMB
Stool	Male	Gastroenteritis	S2	Negative	Positive	Positive
Stool	Male	Typhoid	S4	Negative	Positive	Positive
Stool	Female	Medical Test	S10	Negative	Positive	Positive
Stool	Female	Medical Test	S11	Negative	Positive	Positive
Stool	Female	RVDX	S12	Negative	Positive	Positive
Stool	Female	Medical Test	S17	Negative	Positive	Positive
Urine	Male	Urinary Tract Infection	U3	Negative	Positive	Positive
Urine	Female	Urinary Tract Infection	U12	Negative	Positive	Positive
Urine	Male	BAA to CAP	U7b	Negative	Positive	Positive
Urine	Female	Medical test	U9b	Negative	Positive	Positive
Urine	Female	Urinary Tract Infection	U17c	Negative	Positive	Positive

Table 4 Biochemical characterization of isolates from clinical source.

Isolates	S2	S4	S10	S11	S12	S17	U3	U12	U7b	U9b	U17c
Hydrogen Sulphide	-	-	-	-	-	-	-	-	-	-	-
Indole	+	+	+	-	-	+	+	-	-	-	-
Motility	+	+	+	+	-	-	+	-	+	+	+
Methyl Red	+	+	+	+	+	+	+	+	+	+	+
Voges Proskauer	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	-	+	-	+	+	-
De-Mannitol	+	+	+	+	-	-	+	-	+	+	+
Sucrose	+	-	+	+	-	-	-	-	+	+	+

The chart showing the susceptibility of the eleven isolates of *E. coli* is presented in **Table 5**. In **Table 6**, the frequency of the antibiotic susceptibility of the isolates is presented. 100% of the *E. coli* isolates were resistant to augmentin, gentamycin, Streptomycin, chloramphenicol and tetracycline [13].

The intermediate and susceptibility rates of pefloxacin were 9.09% and 36.36% respectively. Ciproflaxin had a resistance rate of 63.63% and susceptibility rate of 36.36%. **Table 7** shows the multiple resistance patterns of the isolates. 54.54% were resistant to four classes of the five classes of antibiotics used (beta lactam, aminoglycoside, sulphonamide, trimethoprim and macrolide). 46.46% were resistant to all the five classes of antibiotics used (aminoglycoside, sulphonamide, trimethoprim and fluoroquinolone) [14].

Of the 41 samples collected, only 11 isolates were obtained (15.94%). This is slightly higher than the results reported by Kibret and Abera on antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *E. coli* was not isolated from some stool samples because the individuals had been on antibiotics prior sample collection and this may have caused an imbalance in the normal flora of the body. This is in agreement with the reports of Reynolds [13].

In this study, *E. coli* infection was more prominent among females compared to male subjects with ratios 1:1, 1:2, 0:1, 1:3, 1:0, and of ages 11-20, 21-30, 31-40, 41-50, 61-60 respectively. This is in agreement with the findings of Harper and George who researched on female urinary tract infection [14,15]. The highest number of infections in this study was linked to females with age ranges between 21-30 years and 41-50 years.

It could therefore be inferred that *E. coli* infections are not limited to the very young and very old but spread across every age bracket. This further agrees with the reports of Kaper who

reviewed the pathogenesis, epidemiology, diagnosis and clinical aspects of diarrheagenic *E. coli* [16].

Table 5 Antibiotic susceptibility profile of *Escherichia coli.*

Code sample	Augmentin	Gentamycin	Pefloxacin	Oflaxacin	Streptomycin	Tetracycline	Chloramphenicol	Sparfloxacin	Ciprofloxacin	Amoxycillin
S2	R	R	R	R	R	R	R	R	R	R
S4	R	R	R	R	R	R	R	R	I	R
S10	R	R	I	R	R	R	R	I	I	S
S11	R	R	S	R	R	R	R	R	I	R
S12	R	R	S	S	R	R	R	R	I	R
S17	R	R	R	R	R	R	R	R	R	R
U3	R	R	R	R	R	R	R	R	R	R
U12	R	R	R	R	R	R	R	R	R	R
U7b	R	R	S	R	R	R	R	R	R	R
U9b	R	R	S	R	R	R	R	R	R	R
U17c	R	R	R	R	R	R	R	R	R	R
Key: R-Resistance; I-Intermediate; S-Susceptible										

Table 6 Percentage distribution of antibiotic susceptibility of *E. coli* isolated.

Antibiotics with disc potency	Frequency %			
	Resistance	Intermediate	Susceptible	Total
Augmentin 30 µg	100%	0	0	100%
Gentamycin 30 µg	100%	0	0	100%
Pefloxacin 30 µg	54.54%	9.09%	36.36%	100%
Oflaxacin 10 µg	90.90%	0	9.09%	100%
Streptomycin 30 µg	100%	0	0	100%
Tetracycline 30 µg	100%	0	0	100%
Chloramphenicol 30 µg	100%	0	0	100%
Sparfloxacin 10 µg	90.9.%	9.09%	0	100%
Ciprofloxacin 5 µg	63.63%	36.36%	0	100%
Amoxycillin 30 µg	90.90%	0	9.09%	100%

Antimicrobial resistance in *E. coli* has increased worldwide. In this study, the overall resistance of *E. coli* to the selected antimicrobials was high. This result is consistent with the findings of previous studies of Kibret and Abera [7,17].

Based on this study, *E. coli* showed the greatest resistance (100%) to augmentin, gentamycin, streptomycin, tetracycline, and chloramphenicol. This doesn't corroborate the work done by Waseem et al. [18] but is slightly lesser compared to the work of Ogidi and Oyetayo [12].

Table 7 Multiple Antibiotic Resistance pattern of the *E. coli* isolates

No of Antibiotics (Classes)	Antibiotic resistance pattern	Frequency (%)	Total number (%)
4	AUG/GEN/TET/CHL	6(54.54%)	6
5	AUG/GEN/PRF/TET/CHL	5(46.46%)	5
Total		11(100%)	11

The isolates were 100% resistant to augmentin, gentamycin, streptomycin, tetracycline, and chloramphenicol. Less resistance (90.9%) was shown to oflaxin, sparfloxin and amoxycillin used. The least resistance was shown to Ciprofloxacin (63.6%) and Pefloxacin (54.5%). These antibiotics seem effective against the isolated *E. coli.* This corroborates the work done by Onuoha [14] on antibiotics susceptibility pattern of *Escherichia coli* isolated from well water in Afikpo, south eastern Nigeria.

Additionally, Mydryk [9]; Toth et al. [17]; Cox and Wright [4] have all previously reported bacterial resistance against ampicillin, gentamicin, erythromycin, tetracycline and ciprofloxacin at different times. The reason why some of these *E. coli* isolates showed high level of resistance to the antimicrobial agents used is an indication that these antibiotics have been abused, hence the possibility they have acquired resistance. It

has been reported that bacteria acquire resistance by horizontal gene transfer of mobile genetic element and that gross usage of the antibiotics influences the selection of existing resistance mechanisms (Stoke and Gillings, 2011).

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