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Resistance Profile and Plasmid Characteristics in MDR Escherichia Coli Isolates from Patients with Gut and Urinary Tract Infections in Lafia, Nigeria

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***Abstract*—** *Escherichia coli* is a prevalent microorganism responsible for gastrointestinal (GIT) and urinary tract infections (UTIs) in patients attending health facilities all over the world. The study determined the plasmids profile of multi-drug resistant (MDR) *E. coli* from urine and fecal samples. Of the 300 samples analyzed using microbiological techniques, *E. coli* identified were screened for antibiotic susceptibility, and the plasmid DNA of resistant isolates was extracted and amplified on PCR. Obtained *E. coli* were mostly resistant to ampicillin (79.2%) in stool and 82.0% in urine samples while imipenem had the best activity against the bacterium 18.0% (urine) and 9.4% in stool samples. Fifteen resistance patterns were obtained from the different classes of antibiotics tested against *E. coli* from the stool and 14 from the urine samples. Isolates U15 and U16 had 3 plasmids each 7000, 4200, 2500, 10000, 3500, and 2000 respectively to make them resistant isolates with the highest number. The study concluded that *E. coli* from fecal and urine samples of patients presenting infections of gastrointestinal and urinary tracts in Lafia, Nigeria contains plasmid DNA responsible for the multi-drug resistance.

Keywords— Antibiotics, genes, infections, plasmid, virulence

I. INTRODUCTION

Among the antibiotics class in use for stemming the threat of infections due to *Escherichia coli* are fluoroquinolones, aminoglycosides, and β-lactams. The antibiotics exert their actions through the prevention of the production of the peptidoglycan layer in the bacterial cell wall (β-lactams), inhibiting two topoisomerase enzymes necessary for DNA replication (fluoroquinolones). Aminoglycosides prevent the synthesis of bacterial proteins by attaching to the 30s subunit in the ribosome, while sulfonamides and trimethoprim alter folic acid production by manipulating the production of dihydrofolate reductase and tetrahydropteric acid production [1, 2]. Among the challenges encountered in the management of antibiotic resistance in the hospital setting is the choice of selection of effective and adequate empiric drug regimens. It is recommended that this line of treatment should follow strict choices based on the individual antibiogram of a health facility because it differs from one facility to another and from place to place [3].

The presence of plasmids in a bacteria cell compounds the choice of antimicrobials in diseases like bacteremia in patients. Antibiotics administered at the onset of infection are usually not effective in curtailing such infections as plasmid confers resistance to such pathogens [4]. Plasmids are double-stranded, round DNA molecules transferred to bacteria cells permanently or otherwise, and can stay as extra genetic particle replicating in the bacteria host where it promotes virulence and resistance characteristics on the bacteria host. A plasmid can confer multiple antibiotic resistance on a bacterium apart from being capable of transfer from one bacterium to another. Resistance to bacteria is conferred by the resistance factors or resistance plasmids, which enhance resistance against a particular antibiotic drug, and the F plasmids known as conjugal plasmids that majorly confer resistance in *Escherichia coli* [5]. Plasmids replicate autonomously and apart from conferring virulence and antibiotic resistance, are capable of initiating and controlling replication and ensuring stable inheritance. Plasmids are grouped according to the functions they perform into five classes; fertility plasmids (conjugates and expresses sexual features), resistance plasmids (confers resistance against antibiotics), col plasmids (code bacteriocins), virulence plasmids (enhances pathogenic characteristics), and metabolic or degradative plasmids (promotes the breakdown of unusual substances). Cephalosporin and β-lactams with β-lactamase enzyme inhibitors combination are choice drugs for treating *E. coli* associated illnesses [6]. Carbapenems are administered as empiric therapy for complications resulting from *Escherichia coli* containing plasmid in patients with renal infection, those on treatment lately and administered cephalosporin, and fluoroquinolones, and patients with a history of past hospitalization, and individuals with

diabetes mellitus and those with underlying liver infections are at risk [4, 7, 8]. Synthesis of the β-lactamase enzyme by *E. coli* promotes resistance to beta-lactam antibiotics. The enzyme deactivates the β-lactam class of antibiotics thus making them inactive against the bacterium. β-lactamase enzymes are all narrow-spectrum enzymes found in some *E. coli* that mediate resistance to aminopenicillin and early generation cephalosporin [9].

Resistance by bacteria continues to bring with it huge costs and demands from the patients and health institutions. The mortality and risks of co-infections increase as more strains pick up plasmids from the environment. The lack of relevant up-to-date information on plasmid profiling of genes in bacteria from intestinal and urinary sources in the study area necessitated this study. The study determined the antibiotic resistance profile and plasmid characteristics of resistant *E. coli* in people suffering from urinary and gut infections in Dalhatu Araf Specialist Hospital (DASH), Lafia Nigeria.

II. METHODOLOGY

a. Ethical clearance

Approval for the investigation was obtained from the Ethical committee of DASH, Lafia, Nigeria (Appendix 1).

b. Sample size and collection

One hundred and fifty fecal samples and 150 samples of urine were obtained from patients with signs of gut infections and urinary tract infection in DASH, Lafia, Nigeria. According to an earlier study, a prevalence rate of 22.6% was adopted [10]. The collection and processing of samples followed the standard in the 1964 declaration of Helsinki (revised in 2000).

c. Isolation of *E. coli* from urine and fecal samples

Stool samples were inoculated into Deoxychocolate agar (DCA) and urine samples into CLED agar, incubated for 24 h at 37°C. Isolates that ferment lactose had pink color, while non-lactose fermenting colonies appeared whitish on DCA and CLED agar plates. The colonies were enumerated and subcultured on MacConkey and CLED agar to obtain pure *Escherichia coli*. Presumptive identification of *E. coli* was by inoculating new plates containing EMB agar from colonies that had pinkish coloration and colonies that produce greenish metallic sheen were presumptive for *Escherichia coli*. Identification of presumptive isolates followed the methods of Cheesebrough [11].

d. Antibiotic susceptibility test

Susceptibility of isolates to antibiotics was done using the guideline of CLSI [12]. Following the McFarland standard, isolates were inoculated individually into 5 mL 0.8% (w/v) sodium chloride, and the turbidity of the suspensions made to 0.5 McFarland's standard. The suspension (0.02 mL) was inoculated by streaking on Mueller Hilton agar and antibiotics discs placed on it. Incubation was at 37^0C for 24 h following 1 h pre-diffusion. Measurement of the zone of inhibition was taken, and the multiple antibiotic resistance index was calculated (as the number of antibiotics resisted divided by the number of antibiotics used).

e. β-lactamase production by E. coli isolates

The ability to produce β-lactamase enzyme by isolates was confirmed by testing *E. coli* resistance to cefotaxime and ceftazidime with MICs ≥2.0 µg/mL [13]. In the double antibiotic synergy test (DAST), swabs with 105 CFU/mL suspension of the isolates were streaked on Mueller Hinton agar. Amoxicillin-clavulanate (30 µg) and ceftazidime (30 µg) and cefotaxime (30 µg) discs were placed 20 mm away from each other on the plate. The plates after 1 h pre-diffusion were incubated for 24 h at 37^oC. Isolates with clear zones of inhibition to ceftazidime and cefotaxime were confirmed as ESBL producers.

f. Molecular characterization of Escherichia coli

Resistant *E. coli* colonies were introduced into separate Luria-Bertani (LB) broth medium and incubated for 8 h at 37ºC. After incubation, 5 mL of the suspension was centrifuged for 3 min at 14000 rpm. The resulting bacterial cell was dissolved in saline solution (500 µL), heated (95ºC) for 20 min, cooled, and re-centrifuged at the same rpm. The obtained DNA was amplified with a multiplex PCR (Thermo Scientific TM, USA) using cycling conditions of 95°C for 5 min, 94°C for 20 s, 55°C at 30s, and 72°C for 30 s for 30 cycles, with a final 7 min extension at 72°C [14]. The PCR products were run on 1% agarose gel and visualized on a UV Tran illuminator.

g. Preparation and purification of total DNA for plasmid sizes

Isolates with resistant characteristics were analyzed for plasmid DNA adopting the guideline of Schultz et al. [15] and Ojeniyi et al. [16]. Into Luria-Betarni (LB) broth medium (10 mL) was inoculated resistant isolate, incubated at 37°C for 16 h with constant shaking at 250 rpm. Grown bacterial cells were harvested and spun at 8000 rpm for 2 min at 37ºC. The resulting pellets were re-suspended in 250 µL of re-suspension solution and transferred to microcentrifuge tubes and 250 µL of the lysing solution was added, mixed by inversion of the tube 4-6 times to make the solution viscous and clear. About 350 µL of neutralization solution was added and the mixture was inverted, and then centrifuged at 10000 rpm for 5 min to separate cell debris and genomic DNA. The supernatant was transferred to the Gene JET spin column by decanting and centrifuged for 1 min, decanted and washed with 500 µL wash solution then centrifuged again for 30 s. The steps were repeated to completely remove the alcohol after which it was spun in a microcentrifuge tube with 50 µL of elution buffer added to elute plasmid DNA. Incubation was done at room temperature for 2 min and centrifuged for 2 min. The resulting plasmid DNA was stored at -20°C. Quantification of genomic DNA was done on a NanoDrop 1000 spectrophotometer and the DNA fragments were resolved on 1% agarose gel and viewed under a UV transilluminator for interpretation.

III. RESULTS AND DISCUSSION

a) Bacteria counts in urine and stool samples

Ten bacteria genera were obtained from patients' samples collected in the study (Table 1). *Escherichia coli* from urine samples had a percentage bacterial count of 50 (36.5%) followed by *Klebsiella* sp 32 (23.3%), while *E. coli* from the gastrointestinal tract of patients had the highest percentage of the bacterial count of 53 (44.9%). The prevalence of *E. coli* in stool samples was 53 (35.3%), while it was 50 (33.3%) in urine samples. One hundred UTI patients had no *E. coli* isolated from their urine samples, while 97 (64.7%) of stool samples had the bacterium absent in them.

Table 1: Bacteria count in urine and stool samples

Isolates	Stool samples (%)	Urine samples (%) CFU $\geq 10^5$/mL
	N=118	N=137
Escherichia coli	53 (44.9)	50 (36.5)
Klebsiella sp	29 (24.6)	32 (23.3)
Enterococcus sp	-	2 (1.5)
Staphylococcus aureus	-	26 (19.0)
Proteus sp	7 (5.9)	9 (6.6)
Pseudomonas sp	15 (12.7)	13 (9.5)
Streptococcus sp	-	5 (3.6)
Clostridia sp	2 (1.7)	-
Shigella sp	1 (0.9)	-
Salmonella sp	11 (9.3)	-

N= Total number of urine samples that yielded growth

b) Antibiotic susceptibility profile of *Escherichia coli*

The antibiotic susceptibility of *E. coli* isolates in the study showed that ampicillin was the most resisted antibiotic (82% in urine samples and 79.2% in fecal samples) as shown in Table 2. About 18% of the isolates from urine samples were resistant to imipenem as the least resisted antibiotics in the study. Only five isolates were resistant to imipenem from the stool sample collected.

Table 2: Antibiotics susceptibility profile of *Escherichia coli* in patients

S/N	Antibiotic Treatment	Urine (N=50)	Stool (N=53)
1	Ampicillin (30ug)	41 (82%)	42 (79.2%)
2	Amoxicillin/clavulanic acid (30ug)	28 (56%)	34 (64.1%)
3	Ciprofloxacin (5ug)	10 (20%)	12 (22.6%)
4	Ceftazidime (30ug)	19 (38%)	13 (24.5%)
5	Cefotaxime (30ug)	18 (36%)	17 (32.1%)
6	Ceftriaxone (30ug)	10 (20%)	21 (39.6%)
7	Gentamycin (10ug)	27 (54%)	13 (24.5%)
8	Imipenem (30ug)	9 (18%)	5 (9.4%)
9	Streptomycin (30ug)	27 (54%)	23 (43.4%)
10	Sulphamethoxazole/trimethoprim (25ug)	34 (68%)	26 (49.1%)

c) Antibiotics resistance pattern in *E. coli* from patients

The class of antibiotics (50 isolates) was the most resisted group in the study, followed by the penicillins (49 isolates), and the sulphonamides (46) (Table 3). The carbapenems were the most potent as it was resisted by only 14 isolates in the whole study. About 35 resistance patterns were got in the isolates from the two samples (Appendix II).

Table 3: Resistance pattern in isolated *E. coli*

	Stool samples			Urine samples		
S/N	*E. coli* ID	**Antibiotic class resisted**	**MAR Index**	**Isolate**	**Antibiotic class resisted**	**MAR Index**
1	F3, F10	A, X	0.3	U1	A, S	0.3
2	F4, F6, F7	A, P	0.3	U2, U7, U9	A, X, S	0.5
3	F5, F22	X, S, P	0.5	U3, U14, U18, U19	A, X, S, P	0.7
4	F8, F25, F27	A, S, P	0.5	U4, U26	A, S, P	0.5
5	F9, F26, F30	A, X, P	0.5	U5	A, C, X, P	0.7
6	F11, F13	C, X, S	0.5	U6	C, X, S, P	0.7
7	F12, F23	A, X, S	0.5	U8	C, F, X, S	0.7
8	F14	F, X, S, P	0.7	U10	A, C, X, S	0.7
9	F15	A, C, P	0.5	U11, U16	F, X, S, P	0.7
10	F16, F19, F20	F, X, P	0.5	U12	A, C, S, P	0.7
11	F17, F21, F24, F28, F31, F33	A, X, S, P	0.7	U13, U22, U24	A, F, X, P	0.7
12	F18	A, C, S, P	0.7	U15, U20, U21	A, C, X, S, P	0.8
13	F29	A, F, X, S	0.7	U17, U29, U30	A, F, X, S, P	0.8
14	F32	C, F, X, P	0.7	U27	A, C, F, S, P	0.8
15	F34	A, F, X, S, P	0.8			

A – aminoglycosides, C – carbapenems, F – fluoroquinolones, X – cephalosporins, S – sulphonamides, P – penicillins

d) β-lactamase (ESBL) producing *Escherichia coli* isolates

Thirty-eight (76%) isolates from urine were ESBL producers, while 24 (45.3%) from stool samples were ESBL producers of the 50 and 53 *E. coli* isolates respectively.

e) Plasmid characteristics of *Escherichia coli* from patients

Twelve (12) *E. coli* isolates had plasmids isolated from them (Table 4, Plate 1). Isolate U16 (urine) had the highest molecular size of 10000bp, while isolates S7, S8, and S13 (all stool isolates) each had only one plasmid in them.

Table 4: Plasmid characteristics of resistant isolates from patients

E. coli ID	Number of copies	Molecular size (bp)
F2	2	4200, 2200
F7	1	4200
F8	1	3500
F13	1	2500
F21	2	4200, 2250
F22	2	4200, 3500
U4	2	4750, 4200
U7	2	7000, 5000
U14	2	8750, 5000
U15	3	7000, 4200, 2500
U16	3	10000, 3500, 2000
U17	2	7000, 3500

Key: F – Isolate from the fecal sample; U – Isolates from the urine sample

Lane L: Supercoil DNA ladder composed of DNA fragments (in base pairs): 10,000 (A), 7500 (B), 5000 (C), 3500 (D), 2500 (E), 2000 (F). Lane 1-12: *Escherichia coli* isolates; S2, S7, S8, S13, S21, S22, U4, U7, U14, U15, U16, and U17 respectively.

Plate 1: Agarose Gel Electrophoresis of Plasmid DNAs from the *Escherichia coli* isolates

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Discussion

Escherichia coli has been identified to switch from being a commensal to being pathogenic causing infections like typhoid fever and diarrhea. The bacterium promotes its virulence through the production of toxins, and colonization aided by type 1 fimbria. These factors are obtained through the transfer of genes horizontally between bacteria [10]. The high prevalence rate obtained in the study is associated with poor hygiene, and transmission of nosocomial infections, intake of contaminated food and water that pervade communities in the vicinity where the study was carried out. Urinary and gastrointestinal tract infections in hospitals and communities account for the high number of recorded nosocomial causes and recorded bacteremia in patients [17, 18]. Infections relating to the GIT and UT are designated hospital-acquired or community-acquired infections depending on the factors surrounding them. The high prevalence of *E. coli* from the urine of patients sampled agreed with the reports of Córdoba *et al*. [19]. Ngwai *et al.* [20] and Paniagua-Contreras *et al*. [21] posited that the Enterobacteriaceae is the major precursor of acute and uncomplicated UTI in patients. All these bacteria were identified in-patient who participated in the study; and about 90.0% of cases of UTIs in Nigeria had these bacteria isolated from patients [22, 23]. The report presented on these organisms in our study agreed with that of WHO [24].

The study noted that isolates were susceptible to antibiotics cefotaxime, ciprofloxacin, gentamycin, ceftazidime, and imipenem and this was expected as these are expensive antibiotics, not commonly prescribed by clinicians and so could not be readily abused by patients. The susceptibility recorded could be the result of environmental factors and the timing of the experiments as these two factors could vary the results obtained [25]. Another reason adduced for the susceptibility to ceftazidime, cefotaxime, imipenem, and ciprofloxacin was the high cost of obtaining them. Resistance recorded against other antibiotics resulted in part from misuse, abuse, and readily available cheap and substandard forms. Another possible cause is the high rate of consumption of antibiotic-treated poultry animals from where residual consumption of meat could lead to resistance as asserted by Ghafourian *et al*. [26], and Ahmed *et al*. [27]. Continued abuse and misuse of antibiotics could lead to the production of extended-spectrum beta-lactamase enzyme in *E. coli* as seen in our study; other reasons could be through acquisition from the environment including consumption of poorly prepared poultry birds, from the hospital environment, or patients' host communities [10, 28]. *E. coli* isolates from samples collected from patients in the study, harbored plasmid genes that could mediate resistance to antibiotics shown by the isolates.

The study of Ferdous *et al*. [29] and that of Flokas *et al*. [2] who isolated plasmids from *E. coli* isolates from GIT and UT of infected patients in a Lagos hospital in Nigeria corroborated the presence of plasmid genes in the isolates in this study. The duo also posited that the bacterium harbors virulent markers; and pathotypes genes that were multidrug-resistant. Plasmid size affects electrotransformations in bacteria cells. The higher the plasmid size, the lower the optimal field strength which can translate to a reduced survival rate of the bacterium cell [30]. Ohse *et al*. [31] explained that at 2.9 to 12.6 kbp, transformation efficiency (transformants per microgram plasmid DNA) decreased with increases in the size of the DNA. The more the number of plasmids the higher the stability of the bacterial cell, though it is associated with a high metabolic burden.

IV. Conclusion and Future Scope

The study concludes that *E. coli* strains from UT and GIT infected patients attending DASH, Lafia were resistant to ampicillin and amoxicillin, that multi-drug resistant *E. coli* pathogens (isolates) harbored plasmid DNAs genes that might be responsible for the drug resistance observed which are transferrable and spread to other bacteria in the GIT and UT of such patients. Fluoroquinolones and cephalosporins were not very promising in inhibiting the bacterium in the study but unfortunately, these are choice drugs administered by clinicians for the treatment of enteric and urinary tract infections in Nasarawa State, Nigeria. The study suggests further work in determining the prevalence of genes conferring antimicrobial resistance in *E. coli* and other Enterobacteriaceae, and the routes of transmission to understand the means of curtailing the challenge.

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