Characteristics of Virulence Factors and Prevalence of Virulence Markers in Resistant *Escherichia coli* from Patients with Gut and Urinary Infections in Lafia, Nigeria

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ABSTRACT: The spread and transfer of resistant pathogens is on the increase worldwide and it is presently a cause of concern for health facilities, health organizations and governments. Pathogenicity is a factor dependent on the virulence of the microorganisms. The study aimed at determining the virulence markers and factors in multidrug resistant (MDR) *Escherichia coli* isolated from patients with urinary tract and gastrointestinal tract infections in Lafia, Nigeria. Collection of urine and stool samples (150 each) from patients was carried out, and bacteria isolated from the samples using the spread plate technique. Antibiotic susceptibility test was determined to identify resistant *E. coli* isolates after which, virulence factors and genes conferring virulence evaluated. The prevalence of *E. coli* was 33.3% and 35.3% in urine and stool respectively with 42 of the isolates being MDR. All the isolates showed cell surface hydrophobicity on ammonia sulfate molarity at >1.5, and all possessed capacity to produce hemolysin and pyrogen, though isolate U6 produced the highest amount of hemolysin and the other isolates mostly produced reasonable amount of pyrogen. Isolate U19 from urine sample and isolates S6, S10, S11, and S17 from stool samples all had between 81 and 100 serum resistance survival percentages, while 13 of the isolates had no serum resistance capabilities. Virulence conferring genes present in the isolates include *fimH, pap, stb, cs31a, vt2, east1.* Most of the resistant isolates have more than one virulence marker that is a means of producing an effective pathogenesis.

KEYWORDS: Antibiotics, bacteria, pathogenesis, resistance, virulence

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Introduction

Escherichia coli are intestinal commensals that cause gastrointestinal and other-intestinal tract infections in human. The bacterium is one of the leading pathogen responsible for community acquired infections worldwide,[1] with attendant morbidity and associated burden of treatment.[2] Different strains of the bacteria cause nosocomial infections such as gastroenteritis, urinary tract infection (UTI), meningitis, peritonitis, septicemia, cholecytitis, bacteremia in human being and other clinical infection such as neonatal meningitis and pneumonia.[3] The pathogen when resistant to antibiotics leads to complications that cause treatment failure and increase the rate of mortality and morbidity among patients.[4] Antibiotic resistance is a key public challenge in the health sector worldwide,[5] and *E. coli* isolates exhibit multi-drug resistance (MDR) and extensively drug resistance capabilities.[6]

The ease with which the bacterium acquires and losses gene through rearrangement and acquisition make it versatile at blending into hybrid strains with resistance capabilities to known antibiotics thus endowing it with new characteristics.[7] *E. coli* strains invade and colonize inner layers of the intestinal lining causing infections with different clinical symptoms because of the possession of different virulence markers that confer enteroaggressive or uropathogenic characteristics on such isolates. The acquired resistance is mediated by resistant genes located on mobile genetic elements which with ease can be transmitted from the resistant strain to another bacteria.[8] Some mobile plasmids harbor genes that confer resistance to antibiotics and speed up its transmission and spread,[9] and extended spectrum β-lactamases (ESBL) is an example located on an extrachromosomal plasmid DNA.

Bacteria produces adhesin, exoenzymes, toxins, and enzymes that are all virulence agents employed to aid colonization, invasion, pathogenesis, resistance to antibiotics, and severity of infection.[10] Invasiveness can be promoted by evading the host's immune system using serum resistance mechanism and special proteins such as M protein that prevent binding of complement molecules thereby inhibiting phagocytosis, and the use some destructive enzymes. The present study is a surveillance research intended to give up to date data of resistance profile and genes encoding virulence in the urinogenital clinic of Dalhatu Araf Specialist Hospital, Lafia Nigeria. The study was to evaluate virulence factors and determine the prevalence of virulence encoding genes and characteristics of virulence factors in MDR *Escherichia coli* from patients with urinary and gastrointestinal tract infections in Lafia, Nigeria that can aid pathogenesis and disease severity

Materials and Methods

Sample size and collection

Three hundred samples (150 stool and 150 urine) were randomly collected from willing patients with symptoms of urinary tract (UT) and gastrointestinal (GI) tract infections in Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria. Written consent was obtained from the Ethics Committee of Dalhatu Araf Specialist Hospital (DASH) Lafia, Nasarawa State, Nigeria, after which informed written consent was sought from the patients prior to sample collection. Inclusion criteria are male and female adult patients (aged 18-65), on admission in both medical and surgical ward with symptoms of urinary tract (UT) and gastrointestinal (GI) tract infections who gave their consent to participate in the study. Exclusion criteria are patients on admission without urinary tract (UT) and gastrointestinal (GI) tract infections and do not give their consent to be involved in the study.

Isolation and identification of Escherichia coli

Escherichia coli were isolated from the samples by inoculating stool samples on Deoxycholate agar (DCA) and urine samples on Cysteine lactose electrolyte deficient (CLED) agar and Chocolate Agar respectively. The inoculated cultures were incubated for 24 h at 37°C and obtained *E. coli* colonies sub cultured on DCA, MacConkey, and CLED agar plates and incubated for 24 h at 37°C to obtained pure isolates and further inoculated on EMB agar at 37°C for 24 h. Greenish metallic sheen colonies on EMB medium were presumptive for *Escherichia coli.* Identification of *Escherichia coli* was determined using biochemical tests (Indole test, Methyl Red Test/Voges-Proskauer test, Citrate test, and Sugar utilization tests) and their colonial characteristics.

Antibiotic susceptibility test

The antibiotic susceptibility test for isolates were according to the method described by the Clinical and Laboratory Standard Institute.[11] Three colonies of *E. coli* was inoculated separately into 5 mL sterile 0.8% (w/v) sodium chloride (NaCl) (normal saline) and the turbidity of the bacteria suspensions adjusted to the turbidity equivalent of 0.5 McFarlands' standard. A sterile swab stick was soaked in standardized bacterial suspension and streaked on Mueller Hilton agar plates. Antibiotics disk was placed at the center of the plate, allowed to stand for 1 h to prediffuse, and incubated at 37°C for 24 h. The diameter of zones of inhibition in millimeter was measured and the result of the susceptibility test was interpreted in accordance with Clinical and Laboratory Standard Institute.

Evaluation of virulence markers in E. coli *isolates*

Salt aggregation test. The salt aggregation test for cell surface hydrophobicity was carried out adopting the modified methods of Ljungh and Wadström[12] as follows; 10 µL of bacterial suspension made in phosphate buffer was mixed with 10 µL of ammonium sulfate solutions of different molarity (0.01-1.5M) on clean grease free glass slide and observed for 60 seconds while rotating. The highest dilution of solution giving visible clumping of bacteria was scored as salt aggregation positive. Strains showing aggregation in 0.002M phosphate buffer clone (pH 6.8) were considered auto aggregative. Isolates that were with salt aggregation test value ⩽1.3M are considered hydrophobic.

Serum resistance assay. Serum resistance assay was carried out according to the methods of Kumar and Mathur.[13] Two milliliter of 24 h Mueller-Hilton broth (MHB) containing the MDR *E. coli* isolates was centrifuged, washed 3 times with 0.85% NaCl (normal saline) and re-suspended in 4 mL of sterile MHB. Ten microliter of the re-suspended culture was added to 2 mL of 10% serum in Hank's Balance salt solution (HBSS: 137M NaCl, 5:4M KCl, 0.24, 0.44, 1.3, 1.0, 4.2,and 5.6 mM d-glucose, 0.02% Phenol red, distilled to 1000 mL, membrane-filter [per-size 0.45 µm]). Sample containing individual isolate was incubated at 37°C for 24 h. The mixture was diluted in normal saline and inoculated on MHA in duplicates. Reading was taken at Time = 0 and 2 h after visible counts were obtained after incubation at 37°C for 24 h. The percentage serum survival was determined using the formula;

$$\begin{aligned}\text{Percentage serum survival} &= CFU_{tn} / CFU_{t0} \times 100.CFU_{t0}\\ &= \text{number of CFU at T}\\ &= 0 \text{ h and } CFU_{tn}\\ &= \text{number of CFU T} = 2\text{h}\end{aligned}$$

Hemolysin production. Hemolysin production was assayed as follow: pure colonies of *Escherichia coli* was streaked in 5% sheep blood agar and incubated at 37°C for 24 h. Formation of wider zone (halo) around the colonies of *E coli* in 5% sheep blood agar was scored as hemolysin positive. The degree of hemolysis (halo) were scored as + and – for hemolysin production and non-production respectively.

Pyrogen detection in Escherichia coli. The presence of endotoxins (pyrogens) in the isolates was carried out using Pyroster™ ES-F (Wako Chemicals USA) following manufacturer's instructions. The reagents were added to microtiter wells and 2 µL of the sample was added and mixed accordingly. The result was read in a plate reader that measures the intensity of color change over time. The rate of color change proportional to the amount of endotoxins produced by the isolate. Very deep color change (+++), deep color change (++), and low intensity color change (+).

Molecular characterization of virulence markers

Primers. Primers, their sequences and amplicon sizes used to determine the genotypic virulence characteristics of the isolated *E. coli* strains and their prevalence are listed in Table 1 below;

Table 1. Primer sequences for the virulence markers checked.

GENE		SEQUENCE (5′ -3′)	AMPLICON (BP)	REFERENCE
Colonization factors				
fimH	Type I fimbriae	TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell[14]
		GCAGTCACCTGCCCTCCGGTA		
cs31a	Coli surface associated	GGGCGCTCTCTCCTTCAAC	402	Bertin et al[15]
		CGCCCTAATTGCTGGCGAC		
Eae	Intimin	GACCCGGCACAAGCATAAGC	384	Yu and Kaper[16]
		CCACCTGCAGCAACAAGAGG		
Toxins				
Stb	Heat-stable enterotoxin b	ATCGCATTTCTTCTTGCATC	172	Blanco et al[17]
		GGGCGCCAAAGCATGCTCC		
vt2	Verotoxin II	GGGCAGTTATTTTGCTGTGGA	386	Ojeniyi et al[18]
		GTATCTGCCTGAAGCGTAA		

Multiplex polymerase chain reaction (PCR) for Escherichia coli *pathotypes and virulence genes*

DNA extraction (boiling method). Colony of the *E. coli* isolate was inoculated into Luria-Bertani (LB) broth and incubated at 37°C for 8 h. Five milliliter of the LB broth culture containing the bacterial isolates was spun at 14 000 rpm for 3 minutes. The cells were re-suspended in 500 µL of normal saline and heated at 95°C for 20 minutes in the heating chamber. The heated bacterial suspension was cooled on ice and spun for 3 minutes at 14 000 rpm. The supernatant containing the DNA was transferred to a 1.5 mL microcentrifuge tubes and stored at –20°C for other subsequent experimentations. The extracted genomic DNA was quantified using the NanoDrop 1000 spectrophotometer by placing a drop (approximately 2 µL) on the sample space and analyzed using the NanoDrop 1000 software. The multiplex PCR assay for DNA extracted from *E. coli* isolates was carried out and DNA templates were subjected to multiplex PCR with specific primers. The multiplex PCR was performed with a 25 µL reaction mixture containing 5 µL of template DNA, 0.2 µL of 18× PCR buffer II, 1.6 µL of a 1.25 mM mixture of deoxynucleoside triphosphates, 1.6 L of 25 mM $MgCl_2$, and 0.1 µL of 5 U of AmpliTaq Gold DNA polymerase (Thermo Scientific™, USA) per µL, and a 0.2 µM concentration of each primer, which was used at a concentration of 0.4 µM. The thermocycling conditions with a Gene Amp PCR system 9700 (AB Applied Biosystem) were as follows: 95°C for 5 minutes, 94°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds for 30 cycles, with a final 7 minutes extension at 72°C as described by Nguyen et al.[19] The PCR products was resolved on a 1% agarose gel at 120 V for 20 minutes and visualized on a UV transilluminator.

Results

Prevalence of Escherichia coli *in urine and stool sample*

Of the 300, samples collected from patients, patients with gastro-intestinal tract infections had a prevalence of 53 (35.3%) cases, those with urinary tracts infection had 50 (33.3%) cases, while about 100 people had no *E. coli* isolated from their urine samples. Forty-two of the isolates were multidrug resistant (20 from urine and 22 from fecal samples). **S** will denote the fecal isolates while urine isolates will be represented as **U**.

Hydrophobicity of resistant E. coli *isolates from urinary and gastrointestinal tracts*

Of the 42 *E. coli* isolates, 10 (23.8%) from stool samples showed cell surface hydrophobicity on ammonia sulfate molarity (M) from 0.01 to >1.5, and U19 from urine sample had 0.03 to >1.5, while 3 (7.1%) S1, S16, and U15 ranged from 0.1 to >1.5 (Table 2). Isolates S6, S10, S11, S15, S17, U2, U3, U10, U11, and U12 all had positive activity at all the concentrations tested.

Serum resistance potential of isolates from the urinary and gastrointestinal tracts

Serum resistance potential of the resistant isolates in Table 3 showed that 4 isolates from urine samples and 6 from fecal samples obtained percentage survival rate between 81% and 100%, while 23 isolates had survival rate below 20%.

Antibiotic resistance profile and production of hemolysin and pyrogen by resistant of E. coli

The antibiotic resistance profile of *Escherichia coli* isolates from urine and stool of patients with symptoms of UT and GI tract infections attending Dalhatu Araf Specialist Hospital, Lafia

Table 2. Cell surface hydrophobicity of isolates from urinary and gastrointestinal tracts.

ESCHERICHIA COLI ISOLATES	SALT AGGREGATION										
	AMMONIUM SULFATE MOLARITY (M)										
	0.01	0.03	0.06	0.1	0.2	0.4	0.5	1.0	1.3	1.5	>1.5
S1, S16, U15	–	–	–	+	+	+	+	+	+	+	+
S2	–	–	–	–	–	+	+	+	+	+	+
S5, S22, U7, U19	–	+	+	+	+	+	+	+	+	+	+
S12, U20	–	–	–	–	–	–	–	–	–	+	+
S20	–	–	–	–	–	–	–	+	+	+	+
S3, S4, S7, S8, S9, S13, S14, S18, S19, S21, U1, U4, U5, U6, U8, U9, U13, U14, U16, U17, U18	–	–	–	–	–	–	–	–	–	–	+
S6, S10, S11,S15, S17, U2, U3, U10, U11, U12	+	+	+	+	+	+	+	+	+	+	+

S, resistant *Escherichia coli* isolates from gastrointestinal tract; U, resistant *Escherichia coli* isolates from urinary tract; +, positive; –, negative.

Table 3. Serum resistance potential of resistant *E. coli* isolates.

PERCENTAGE SURVIVAL	URINE SAMPLE		FECAL SAMPLE	
	ISOLATES	NO OF ISOLATES (%)	ISOLATES	NO OF ISOLATES (%)
		N=20		N=22
0	U1,U4,U5,U13,U14	5 (25.00)	S2,S5,S12,S13,S15,S16,S20,S21	8 (36.36)
1-20	U2,U3,U7,U8,U16,U17	6 (30.00)	S3,S4,S7,S19	4 (18.18)
21-40	U18,U20	2 (10.00)	S8,S9,S14	3 (13.63)
41-60	U9,U11,U12	3 (15.00)	S18	1 (4.54)
61-80	U6,U10,U15	3 (15.00)	S1,S22	2 (9.09)
81-100	U19	1 (5.00)	S6,S10,S11,S17	4 (18.18)

showed that 42 isolates of *E. coli* had Multiple Antibiotics Resistance (MAR) Index of ⩾0.2 as shown in Table 1. Hemolysin production is higher in *Escherichia coli* isolates from urine compared to those from stool (Table 4). Eight isolates from urine samples showed hemolysin production. Twenty-one (50%) of the resistant *Escherichia coli* isolates from stool and urine samples (Table 3) produced high amount (+++) of endotoxin (pyrogen), 15 (35.7%) isolates produced pyrogen moderately (++), while isolates S3, S4, S10, S21, U8, and U15 produced the least (+) endotoxin.

Resistant isolates and their virulence marker genes

A total of 23 *E. coli* isolates contained virulence markers (genes) with 14 (60.9%) from urine and 9 (39.1%) from stool samples (Table 5). *fimH* is present in the 14 *E. coli* isolates from stool samples, while isolates S2, S7, S17, U3, U4, U12, U15, U16, and U17 had *fimH* as the only virulent gene in them respectively.

Discussion

The result obtained in the study showed that *E. coli* was over 30% prevalent in urine and stool samples collected. The high presence of the organism could be attributed to nosocomial infections, lack of personal hygiene amongst the patients, intake of contaminated water or food that is common in the hospital and many African communities. Other factors include unprotected sexual contact, and improper douching of the anal region in women. The findings in this study is in agreement with the study by Tenaillon et al[20] and Shapiro and Jones.[21] The high resistance of *E. coli* isolates to ampicillin, amoxicillin/clavulanic acid, ceftriaxone, and sulphamethoxazole/trimethoprim could not be unconnected to the cheap cost of these drugs making them readily available and affordable to the public leading to misuse and abuse. Findings from the study showed that isolated *E. coli* strains were susceptible to imipenem, ciprofloxacin, ceftazidine, gentamycin and cefotaxime. The susceptibility recorded of *E. coli* isolates could be due to the costly nature of the antibiotics and environment. Antibiotic

Table 4. Production of virulence factors and the antibiotic resistance profile of *E. coli* isolates.

S/N	*ESCHERICHIA COLI* ISOLATES	ALPHA HEMOLYSIN	PYROGEN DETECTION	ANTIBIOTIC RESISTED
1	S1	-	++	CTX, IMP, SXT, CIP
2	S2	-	+++	CTX, CN, SXT, CAZ
3	S3	x	+	CRO, IMP, SXT, CN
4	S4	-	+	CRO, IMP, SXT
5	S5	-	++	AMP, CIP, CRO, SXT
6	S6	-	+++	AMP, AMC, IMP, STR
7	S7	-	+++	AMP, CIP, CTX, CRO
8	S8	-	+++	AMP, AMC, STR, CAZ, SXT
9	S9	-	++	AMP, CN, IMP, SXT
10	S10	-	+	AMP, AMC, CIP, CRO, CAZ
11	S11	-	++	AMP, AMCCIP, CTX
12	S12	-	++	AMP, STR, SXT, CRO
13	S13	-	+++	AMP, AMC, SXT, CAZ
14	S14	x	++	CRO, CN, STR, SXT
15	S15	-	+++	AMP, CN, SXT, CTX
16	S16	-	+++	AMP, AMC, STR, SXT
17	S17	-	+++	AMP, STR, CAZ, CRO
18	S18	-	++	AMP, AMC, SXT, CN
19	S19	-	++	AMP, STR, CTX, SXT
20	S20	-	+++	SXT, CIP, CAZ, STR
21	S21	-	+	AMC, STR, CTX, CN
22	S22	-	+++	AMP, AMC, STR, SXT, CRO
23	U1	x	+++	AMP, AMC, CIP, IMP, CRO
24	U2	-	+++	AMP, AMC, CN, SXT, CTX
25	U3	-	+++	AMP, AMC, CAZ, CRO, SXT, CIP, STR, CT
26	U4	-	++	AMP, CN, SXT, CTX, IMP
27	U5	-	+++	AMP, STR, CAZ, CN, CIP
28	U6	x	+++	AMP, STR, CTX, SXT, CN
29	U7	-	+++	SXT, CIP, CAZ, STR, CN
30	U8	x	+	AMC, CTX, CN, SXT, CAZ
31	U9	x	++	AMP, AMC, STR, SXT, CN
32	U10	x	+++	AMP, CIP, IMP, SXT, CN
33	U11	-	++	AMP, AMC, CN, SXT, CTX, CAZ

(Continued)

Table 4. (Continued)

S/N	*ESCHERICHIA COLI* ISOLATES	ALPHA HEMOLYSIN	PYROGEN DETECTION	ANTIBIOTIC RESISTED
34	U12	-	++	AMP, CAZ, SXT, CIP, STR, CTX, CN
35	U13	-	++	AMP, STR, CTX, SXT, CN
36	U14	x	+++	SXT, CIP, CAZ, STR, CN
37	U15	-	+	AMC, CTX, CN, SXT, CAZ
38	U16	-	+++	AMP, AMC, STR, SXT, CN
39	U17	-	++	AMP, CIP, IMP, SXT, CN
40	U18	-	++	AMP, AMC, CN, SXT, CTX, CAZ
41	U19	x	+++	AMP, CAZ, SXT, CIP, STR, CTX, CN
42	U20	x	+++	AMP, AMC, CIP, CAZ, CN, STR, SXT, CTX

x, hemolysin producer; -, non-hemolysin producer; +++, very deep color change; ++, deep color change; +, low intensity color change; S, resistant *Escherichia coli* isolates from gastrointestinal tract; U, resistant *Escherichia coli* isolates from urinary tract; IPM, imipenems; CIP, ciprofloxacin; CAZ, ceftazidine; CN, gentamycin; CTX, cefotaxime; CRO, ceftriaxone; STR, streptomycin; SXT, sulphamethoxazole/trimethoprim; AMC, amoxycillin/clavulanic acid; AMP, ampicillin.

Table 5. Prevalence of genes encoding virulence markers in *Escherichia coli* isolates.

VIRULENCE MARKERS	NO. OF ISOLATES	ISOLATES
fimH	23	S2, S5, S7, S8, S11, S14, S17, S19, S21, U1, U2, U3, U4, U5, U6, U7, U9, U12, U13, U15, U16, U17, U20
pap	4	S5, S11, S14, U6
stb	3	S8, U2, U13
cs31a	3	U5, U7, U20
vt2	1	U9
east1	3	S19, S21, U1, U7, U13
Total	36	

S, isolates from gastrointestinal tract; U, isolates from urinary tract.

susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment.[20,22]

All the 42 isolates showed varying levels of surface hydrophobicity, which was partly in agreement with the earlier study of Sharma et al[23] who obtained 27.6% isolates that were surface hydrophobic positive. Surface hydrophobicity is a physicochemical property associated with successful adhesion and colonization of a niche by bacteria. Adhesion by the resistant *E. coli* isolates is possible through hydrophobic interactions of (C-(C,H)) functional groups between surfaces of the host cell and bacterial cell which leads to biofilm formation and damage of the surface that leads to the emergence of infection. Serum resistance is the characteristic possessed by a pathogen to help it overcome the phagocytic and destructive activities of the serum system and its components. This bacterial potential is important to be able to overcome the lytic action of serum. Findings in this study showed that 75% *E. coli* isolates from the UT had capacity to resist and possibly overcome serum killing, while 63.63% GIT isolates possess the same potential. Sharma et al[23] proposed that bacteria that possess the attribute of serum resistance could better invade and survive in the human blood stream. Isolates with over 61% serum survival capabilities obtained in the present study could have evolved due to the presence of plasmid, which increase serum resistance in bacteria.[24] Serum resistance exhibited by *E. coli* could be capsule induced. The capsule contain capsular (K) antigen peculiar to some strains of *E. coli* confers serum resistance capabilities on the pathogen, while the polysaccharide layer is composed of O-antigen which dislodges antibodies by blocking binding sites against complement.

About 10 of the isolates from UTI and GIT patients had capacities to produce hemolysin. Hemolysin is a pore-forming toxin that cause cell membrane injury and cell death. It is a broad spectrum endotoxin that increases severity of infection through induction of hemolysis, apoptosis and promotion of nephropathogenicity by exfoliating bladder epithelial.[25,26] While 23.8% of resistant *E. coli* strains presented hemolytic activities, production and exhibition of hemolytic activity by *E. coli* strains is a factor of differential genetic expression.[27-29] Hemolysin production and its virulence potential is strain specific and correlate with increased invasiveness and severity.[29,30] Pyrogen is another virulence factor that induces fever, shock and causes changes in the physiological functioning of the host targeted cells. About 85.71% of the MDR *E. coli* isolates were producers of pyrogen, which is a toxic substance, produced when the bacteria cell undergo binary fission or when the it

dies. At death, the cell disintegrate to release the lipopolysaccharide on its outer membrane into the surrounding cells thus causing necrosis, which triggers the immune system inflammatory response.

The *fimH* gene encodes fimbriae as a colonizing factor that damages the urinary tract epithelial cells by promoting inflammation. The gene was harbored in the DNA of 54.76% of the *E. coli* isolates, 4 isolates had *pap* gene, and 3 *E. coli* isolates had *cs31a* gene. The genes promotes adhesion and make pathogenicity more effective and successful. *Pap* gene encoding fimbriae promote colonization of proximal and distal tubular cells,[31] while *cs31a* gene is an adhesion factor commonly found in animals. Colonization of cell surface by *E. coli* strain is dependent on the expression of different fimbrial adhesion encoded by Pathogenicity-Associated Islands (PAIs), and it encodes different virulent factor.[32] The identified genes in the *E. coli* isolates likely resulted from acquisition from other microorganisms found within the GIT and UT of the host through gene transfer, or from the hospital environment and the host communities.

Other genes *vt2, stb*, and *east1* in the isolates though present in few isolates are virulence encoding genes promoting circumvention of the host immune system and creating lethal pathogens. While *vt2* encode verotoxin 2 production, *east1* is plasmid encoded, and *stb* is a gene encoding heat stable enterotoxin b. The presence of *stb*, *east1, FimH*, and *cs31a* in the present study collaborated earlier work by Valat et al,[33] Umpierez et al,[34] and Abdulkareem et al.[35] The study also support earlier studies of Doughari et al[36] and Sharma et al[23] that *E. coli* contain multiplicity of virulence factors to help it evade host immune system and still cause disease. Barigye et al[37] explained that the presence of an array of virulence agents do not automatically translate to higher capacity to cause disease or pathogenic.

Conclusion

The study highlights the presence of virulence factors that target attachment, immunoevasion, and immunosuppression processes to weaken and induce infection in the host. A collection of these virulence factors while it could be devastating and efficient in overcoming the host defense system is also a veritable option that can be explored as a means of infection control and management. *E. coli* strains with different virulence genes isolated from urine and stool in the present study can cause bacterial infections. Rearrangement of genes and acquisition of genes by pathogen allows the emergent of isolates with an array of virulent genes and resistance to antimicrobial substances that increases disease burden. The study recommends proper hygiene and strict adherence to physician's directive in the use of antibiotics to forestall further resistance

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Authors' Contributions

OOO designed the study, analyzed samples data, and wrote the final draft of the manuscript; SMG collected samples, analyzed them, while VSF did statistical analysis of data obtained and interpreted the data. All the authors read and approved the final copy of the manuscript.

Ethical Approval

The protocol for this study was approved by the Ethical Committee of the Dalhatu Araf Specialist Hospital, Lafia Nasarawa State, Nigeria.

Declarations

We declare that this study is our original work carried out on patients Lafia, Nigeria

Data Availability Statement

All data, machines, experiments, and analysis sources were appropriately acknowledged as necessary while writing the manuscript. Other data generated during the study are attached as supporting documents while any other needed may be available on request.

Supplemental Material

Supplemental material for this article is available online.

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