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Research Article

Phylogenicity and Virulence Profiles of Clinical *Escherichia coli* Isolates in the Ho Teaching Hospital of Ghana

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Background. *Escherichia coli* bacteria are Gram-negative, non-spore-forming aerobes or facultative anaerobic rods. Some strains are pathogenic in men while others are commensals in the gut. The pathogenic strains cause a wide array of diseases by virtue of virulence factors. The commensal strains are generally categorized into phylogenetic groups A and B1. The aim of this study was to determine the association between phylogeny of *E. coli* isolates and virulence and sociodemographic characteristics of the study subjects. *Method*. This study was a cross-sectional study carried out from July 2018 to June 2019. *E. coli* isolates obtained from different clinical specimens were subjected to polymerase chain reaction to determine their phylogenetic groupings and virulence. *Results*. The majority of the isolates belonged to phylogroup A 101 (74.8%), and the predominant virulent gene was fimA (88.9%). There was no significant correlation between phylogenicity and virulence, except for chuA which was found in all isolates that belonged to phylogroups clade I and D. None of the 101 isolates that belonged to group A had the chuA virulence gene. There was a significant association between patient age category and phylogenetic groups B1 and D. *Conclusions*. This study assessed the relationship between the phylogenetic distribution and the virulence profile of clinical isolates of *E. coli*. The virulence of isolates belonging to phylogroup A, which are generally considered as commensals, is alarming. Measures must therefore be put in place to control the spread of these virulent *E. coli*.

1. Introduction

Escherichia coli bacteria are Gram-negative facultative anaerobic rods. They are non-spore-forming and possess peritrichous flagella for motility. These bacteria are potential aetiologic agents of various disease states such as cholecystitis, pneumonia, pyelonephritis, cystitis, urinary tract infection, newborn meningitis, septicaemia, central nervous, and respiratory infections [1]. However, it is worthy of note that many *Escherichia coli* strains are commensal resident in the gut. The barrier between commensalism and virulence of *E. coli* largely depends on a balance between the host immune status and the ability of the bacteria to elicit their virulence factors [2].

Extraintestinal pathogenic *E. coli* is one of the leading causes of morbidity and mortality globally [3]. The degree of pathogenicity of extraintestinal *E. coli* isolates depends on the presence of fimbriae adhesins (*fimA*, *sfa*/*foc*, and *yfcV*) which are virulent factors responsible for adherence. Adherence is a prerequisite for initiating and colonization to specific host cells [4]. Another virulent factors are haemolysins (*hylA and hylF*), which are responsible for lysing red blood cells and human renal epithelial cells. Siderophores (ferric aerobactin receptor (*iutA*), Yersiniabactin receptor (*fyuA*)) also function to sequester iron from the host. Another virulence factor is the cytotoxic necrotizing factor (*cnf1*) which is implicated in tissue damage and dysfunction of a local immune response [5]. Further, the virulence gene

encoding vacuolating toxin (*vat*) is accountable for delaying neutrophil infiltration of the urinary tract in response to uropathogenic *E. coli* by cleaving surface glycoproteins from leukocytes that are involved in neutrophil attraction and migration [6]. These virulence factors contribute significantly to the pathogenicity of *E. coli* strains by colonizing the key anatomical sites, reducing immune response, affecting physiology, and invading host tissues [7].

Before 2013, phylogenetic analysis categorized *E. coli* into four phylogenetic groups: A, B1, B2, and D. This classification was based on a combination of two genes (*chuA* and *yjaA*) and anonymous DNA fragment *TSPE.C2* by use of polymerase chain reaction (PCR) [8]. The commensal *E. coli* strains were associated with phylogenetic groups A and B1 [9, 10]. In contrast, extraintestinal pathogenic *E. coli* strains that carry more virulence genes belonged to phylogenetic groups B2 and D. Extraintestinal infections are mainly due to *E. coli* strains belonging to phylogroup B2 and, to a lesser extent, group D [11]. Other techniques used to determine the phylogenetic groups are multilocus enzyme electrophoresis, ribotyping, random amplified polymorphic DNA analysis, fluorescent amplified-fragment length polymorphism analysis, analysis of variance at mononucleotide repeats in intergenic sequences, and multilocus sequence typing [12].

There has been advancement in the knowledge on the multilocus sequence and genome data resulting in a better understanding of *E. coli* phylogroup classification since 2000. That notwithstanding, the available data at the time showed that 15%–20% of the phylogroups were incorrectly assigned [13]. Further studies have demonstrated some other unclassified strains such as phylogroup E [14], phylogroup F [13], and Escherichia cryptic clade I [8]. This drawback in the classification of the *E. coli* phylogroup necessitated a revised grouping of *E. coli* isolates into eight phylogroups (A, B1, B2, C, D, E, F, and Escherichia clade I) [8].

In Ghana, however, data on the virulence potentials and phylogenetic groupings of extraintestinal *E. coli* isolates are nonexistent. Thus, this study is aimed at detecting the virulence encoding genes of *E. coli* isolates from various clinical specimens and determining their phylogenetic groups in the Ho Teaching Hospital of Ghana.

2. Materials and Methods

2.1. Study Design. This was a cross-sectional study consisting of 135 *E. coli* organisms. The isolates were obtained from various clinical samples produced by 135 patients who visited the Ho Teaching Hospital from July 2018 to June 2019. Majority, 98 (72.6%), of these samples were urine from patients with urinary tract infection. The rest were wound swabs, 14 (10.4%); high vaginal swabs (HVS), 10 (7.4%); blood, 5 (3.7%); ear swabs, 5 (3.7%); sputum, 2 (1.5%), and pleural aspirate, 1 (0.7%).

2.2. The Bacterial Isolates. E. coli isolates were cultured from various clinical specimens, including urine, high vaginal swabs, blood on blood agar, and MacConkey agar (Oxoid, UK). Growths suspected to be *E. coli* were confirmed using the Gram stain reaction, triple sugar fermentation test, citrate test, urease test, indole test, Voges Proskauer, and methyl red test. The identified organisms were inoculated into 80% glycerol-Mueller Hinton broth and stored in a −80°C freezer and later used for other tests. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* NCTC 13442 were used as control organisms.

2.3. Molecular Detection of Virulence Factors and Phylogenetic Groups

2.3.1. Revival of Isolates and DNA Extraction. The isolates were retrieved from the freezer, and the surface was aseptically scraped and emulsified in 30 ml Luria Bertani broth (Oxoid, UK) and incubated overnight in a shaking incubator. Genomic deoxyribonucleic acid (DNA) was extracted from the overnight culture using a high-molecular weight phenol-chloroform extraction method [15], except that Tris-EDTA (TE) was used as the elution buffer. The extracted DNA was incubated at 4°C for two days to resuspend the pellet into a translucent viscous gel. The concentration of the extracted DNA was measured using a NanoDrop spectrophotometer (Thermo Scientific), and the viscous DNA was stored under −80°C.

2.3.2. Detection of Virulence Genes and Phylogenetic Groups by Polymerase Chain Reaction (PCR). Escherichia coli isolates were characterized using primer sequence from previous studies to determine the phylogenetic characteristics (Table 1) and virulence factors (Table 2). The primers were purchased from Integrated DNA Technologies, UK. OneTaq Quick-Load 2x Master Mix with standard buffer purchased from New England Biolabs® was used for the PCR. Lyophilized forward and reverse primers were reconstituted by adding a calculated amount of molecular-grade water using the nmol of the primers to give a concentration of 100 μM. A 1 in 10 working solution was prepared by taking 10 μl of the stock and 90 μl of the molecular-grade water to give 10 μM. A total of 12.5 μl reaction volume was used, comprising 6.25 μl of OneTaq Quick-Load 2x Master Mix with standard buffer, 0.25 μl each of 10 μM forward and reverse primers, 1 μl of template DNA, and 4.75 μl of nuclease-free water.

The initial denaturation and the final elongation for both phylogenetic groups and virulence factors were carried out at 94°C for 30 seconds and 68°C for 5 minutes, respectively. For phylogenetic groups, the PCR conditions were denaturation for 30 seconds at 94°C and annealing for 45 seconds at 55°C (for *chuA* and *arpA*), 51°C (for *yjaA*), 47°C (for *TspE4.C2* and *ArpAgpE*), and 53°C (for *TrpAgpC*).

PCR conditions for the determination of virulence genes were denaturation for 30 seconds at 94°C and annealing for 45 seconds at 49°C (for *ibeA*), 50°C (for *iutA* and *yfcV*), 52°C (for *fimA*), 54°C (for *hylF* and *cnf 1*), 55°C (for *hlyA*, *neuC*, and *chuA*), 57°C (for *sfa*/*foc*), and 58°C (for *vat* and *fyuA*). The initial elongation was done at 68°C for 1 minute/kb.

The annealing temperatures for the various set of primers were calculated using the NEB Tm calculator [19]. In-house DNA (stored positive DNA from previous

Table 1: Primers used in the determination of phylogenetic grouping of *E. coli* isolates.

Target gene	Primer ID	Primer sequences (5′–3′)	Amplicon size (bp)	Reference
chuA	chuA_F	ATGGTACCGGACGAACCAAC	288	[8]
	chuA_R	TGCCGCCAGTACCAAAGACA		
yjaA	yjaA_F	CAAACGTGAAGTGTCAGGAG	211	[8]
	yjaA_R	AATGCGTTCCTCAACCTGTG		
TspE4.C2	TsepC4.C2_F	CACTATTCGTAAGGTCATCC	152	[8]
	TsepC4.C2_R	AGTTTATCGCTGCGGGTCGC		
arpA	arpA_F	AACGCTATTCGCCAGCTTGC	400	[8]
	arpA_R	TCTCCCCATACCGTACGCTA		
ArpAgpE	ArpAgpE_F	GATTCCATCTTGTCAAAATATGCC	301	[16]
	ArpAgpE_R	GAAAAGAAAAAGAATTCCCAAGAG		
TrpAgpC	trpAgpC_F	AGTTTTATGCCCAGTGCGAG	219	[16]
	trpAgpC_R	TCTGCGCCGGTCACGCCC		

Table 2: Oligonucleotide primers used to detect virulence genes.

Target gene	Primer ID	Primer sequence (5′-3′)	Amplicon size (bp)	Reference
ibeA	ibeA_F	TGAACGTTTCGGTTGTTTTG	814	[17]
	ibeA_R	TGTTCAAATCCTGGCTGGAA		
hlyA	hylA_F	AACAAGGATAAGCACTGTTCTGGC	1177	[17]
	hylA_R	ACCATATAAGCGGTCATTCCCGTCA		
hlyF	hylF_F	TCGTTTAGGGTGCTTACCTTCAAC	444	[17]
	hylF_R	TTTGGCGGTTTAGGCATTCC		
neuC	neuC_F	AGGTGAAAAGCCTGGTAGTGTG	676	[17]
	neuC_R	GGTGGTACATCCCGGGATGTC		
iutA	iutA_F	ATGAGCATATCTCCGGACG	587	[17]
	iutA_R	CAGGTCGAAGAACATCTGG		
sfa/foc	sfa/foc_F	CTCCGGAGAACTGGGTGCATCTTAC	410	[17]
	sfa/foc_R	CGGAGGAGTAATTACAAACCTGGCA		
fimA	fimA_F	CGGCTCTGTCCCTSAGT	500	[17]
	fimA_R	GTCGCATCCGCATTAGC		
yfcV	yfcV_F	ACATGGAGACCACGTTCACC	292	[18]
	yfcV_R	GTAATCTGGAATGTGGTCAGG		
cnf 1	cnf 1_F	AAGATGGAGTTTCCTATGCAGGAG	498	[17]
	cnf 1_R	CATTCAGAGTCCTGCCCTCATTATT		
vat	vat_F	TCAGGACACGTTCAGGCATTCAGT	1100	[18]
	Vat_R	GGCCAGAACATTTGCTCCCTTGTT		
fyuA	fyuA_F	F_GTAAACAATCTTCCCGCTCGGCAT	850	[18]
	fyuA_R	R_TGACGATTAACGAACCGGAAGGGA		

experiments) in the laboratory were used as positive controls whereas the Ambion® Nuclease-free Water (Life Technologies, USA) was used as negative control. Thermocycling was done for 30 cycles.

2.3.3. Loading of Amplicons. Using a micropipette, the resulting PCR products were loaded into agarose wells. The first and second lanes were loaded with 6 μl of the either 50 bp, 100 bp, or 1 kb DNA ladder and 10 μl of in-house-generated positive DNA samples. The last lane was loaded with sterile nuclease-free water which served as a negative control test. The remaining lanes were loaded with samples under investigation.

2.4. Statistical Analysis. All analyses were conducted using the SPSS version 25 software and GraphPad Prism 6. Variables were expressed as percentages (%), and the chi-square test was performed to assess the relationships between variables. Variables such as the virulence genes and phylogenetic groups of the *E. coli* strains and

sociodemographic and socioeconomic characteristics of patients were considered for the chi-square analysis. P values < 0.05 were considered statistically significant.

2.5. Ethical Considerations. Ethical clearance for the study was granted by the Joint Committee on Human Research, Publication and Ethics (CHRPE), School of Medical Sciences and Dentistry, and Komfo Anokye Teaching Hospital, with the protocol number CHRPE/AP/204/18.

3. Results

A total of 135 clinical isolates of *E. coli* were recovered from the various clinical specimens of patients who visited the Ho Teaching Hospital for medical care. Only 1 (0.7%) isolate was recovered from the pleural aspirate whereas multiple isolates 98 (72.6%) were recovered from the urine samples of patients with urinary tract infections. The proportion of isolates from the other samples was 14 (10.4%) from wound swabs, 10 (7.4%) from HVS, 5 (3.7%) each from blood and ear swabs, and 2 (1.5%) from sputum. The relationship between sociodemographic and patients' characteristics and the phylogeny of *E. coli* isolates is presented in Table 3. The majority of the isolates (82.2%) came from women, and by the type of patients, the majority was from outpatients (72.6%). There was no significant difference between the proportion of isolates in each phylogroup when compared by gender. Similarly, there was no significant difference between the proportion of phylogroups when compared by the other demographic characteristics (religion, marital status, and occupation), sample type (urine or non-urine), and patient type (out- or in-patient). Age wise, there was no statistically significant difference between the proportion of isolates by phylogroups except for isolates that belonged to phylogroup B1 ($P = 0.017$) and D ($P = 0.007$).

Phylogenetic analysis segregated the 135 *E. coli* isolates into phylogenetic groups A 101 (74.8%), B1 3(2.2%), B2 20 (14.8%), C 4(3.0%), clade I 2(1.5%), and D 5(3.7%).

A total of 12 different virulence genes were identified with the clinical isolates. The virulent gene *fimA* 120 (88.9%) was the most prevalent, while three (3) of the remaining were prevalent in more than 50% of the isolates (*fyuA* 106 (78.5%), *yfcV* 104 (77.0%), and *iutA* 88 (65.2%)). On the other hand, three of the virulence genes under investigation were identified in less than 15% of the total isolates (*chuA* 18 (13.3%), *ibeA* 16 (11.9%), and *hlyF* 7(5.2%)). Other virulence genes identified were *vat* 56 (41.5%), *hylA* 41 (30.4%), *neuC* 40 (29.6%), *cnf 1* 39 (28.9%), and *sfa*/*foc* 37 (27.4%).

Table 3 presents the relationship between sociodemographic and patients' characteristics and the phylogeny of *E. coli* isolates. There is a significant association between patient's age category and phylogenetic groups B1 and D.

Chi-square analysis for the correlation between virulence genes and the phylogenetic groups of isolates showed no correlation between the two except for the virulence gene *ChuA* ($P < 0.0001$) where the proportions of clade 1 and D were higher than those of the others. The distribution also presented that none of the 101 isolates belonging to group A had the chuA virulence gene. Similarly, none of the B1 and C phylogroups possessed the *ChuA* virulence gene. Details of these results can be found in Table 4.

4. Discussion

This study investigated the virulence profile and phylogenetic characteristics of extraintestinal pathogenic *E. coli.* Majority of the organisms were recovered from urine samples received from patients with urinary tract infections. Our finding is consistent with a study by Lara et al. [20] who reported that urinary tract infections are the most common extraintestinal infection caused by *E. coli*. Our study reported that most of the uropathogenic *E. coli* belongs to phylogenetic group A. The preponderance of phylogenetic group A in uropathogenic *E. coli* isolates which is usually associated with commensal strains suggests that the gastrointestinal tract is the main source of strains that colonize the urinary tract [21].

Due to the pathogenicity of *E. coli*, it is an important cause of extraintestinal infections in health facilities around the globe. The *E. coli* organism must first adhere to the host cell to establish infection, and this is achieved by its surface adhesins [22]. The current study investigated the presence of *fimA*, *yfcv*, and sfa/foc virulence genes responsible for adhesions. These genes were detected in 88.9%, 77.0%, and 27.4% of the *E. coli* isolates, respectively. Similar results were reported in other parts of the world for *sfa*/*foc* genes [3, 23] and fimA genes [24, 25]. These plasmid-encoded fimA genes were commonly found in isolates from infection at the lower urinary tract, where they adhere to the urethral mucosa epithelial cells [24].

In the present study, haemolysin-encoding genes, *hlyA* and *hlyF*, were present in 30.4% and 5.2% of the isolates respectively. A higher percentage of *hly*-encoding genes (41.2%) among the *E. coli* isolates was observed in Japan [26]. Contrary to our finding, Agarwal et al. [27] in India reported a lower prevalence of 4.7% of *E. coli* isolates carrying haemolysin genes in women suffering from acute cystitis. *HlyA* is a pore-forming exotoxin secreted to lyse red blood cells and human renal epithelial cells by creating pores in them. The *E. coli* organisms utilize the iron released from the lysed erythrocytes through the siderophore system. Its production and expression are controlled by the availability of iron [28]. A high proportion of *hlyA* recorded in this study belonged to phylogroup D, which corroborates the finding of Zhang et al. [29]. That study reported that highly haemolytic isolates belonged to phylogroup D.

Most of the *E. coli* isolates in this study belonged to phylogenetic group A (74.8%). Our finding contradicted the study by Iranpour et al. [16], who reported phylogenetic group A minority (0.7%). A plausible reason for this observation could be the difference in sample characteristics; while in their study, they used *E. coli* isolates from patients with urinary tract infections, our study involved not only urine samples. Although isolates belonging to this phylogenetic group were generally regarded as commensals in the gastrointestinal tract, they transcended the gut and had access to the urinary tract and caused infections. This could

Table 3: Relationship between sociodemographic characteristics of study participants and the phylogenetic groups of *E. coli*.

Phylogroups	Total	A ($n = 101$)	P value	B1 ($n = 3$)	*P* value	B2 ($n = 20$)	*P* value	C ($n = 4$)	*P* value	Clade 1 ($n = 2$)	*P* value	D ($n = 5$)	*P* value
Sex													
Male	**24**	16 (66.7)	0.311	0 (0.0)	1.000	5 (20.8)	0.353	1 (4.2)	0.547	1 (4.2)	0.325	1 (4.2)	1.000
Female	**111**	85 (76.6)		3 (0.3)		15 (13.5)		3 (2.7)		1 (0.9)		4 (3.6)	
Age													
<10	**11**	7 (63.3)	0.058	2 (18.2)	**0.017**	1 (9.1)	0.064	0 (0.0)	0.154	1 (9.1)	0.386	0 (0.0)	**0.007**
10–19	**2**	0 (0.0)		0 (0.0)		1 (50)		0 (0.0)		0 (0.0)		1 (50)	
20–29	**26**	17 (65.4)		1 (3.9)		7 (26.9)		0 (0.0)		0 (0.0)		1 (3.9)	
30–39	**32**	29 (90.6)		0 (0.0)		2 (6.25)		0 (0.0)		1 (3.1)		0 (0.0)	
40–49	**19**	14 (73.7)		0 (0.0)		5 (26.3)		0 (0.0)		0 (0.0)		0 (0.0)	
50–59	**16**	12 (75.0)		0 (0.0)		0 (0.0)		2 (12.5)		0 (0.0)		2 (12.5)	
≥60	**29**	22 (75.9)		0 (0.0)		4 (14.0)		2 (7.0)		0 (0.0)		1 (3.5)	
Religion													
Christian	**128**	95 (74.2)	0.307	3 (2.3)	0.920	19 (14.8)	0.243	4 (3.1)	0.893	2 (1.6)	0.946	5 (3.9)	0.868
Islam	**2**	1 (50.0)		0 (0.0)		1 (50.0)		0 (0.0)		0 (0.0)		0 (0.0)	
None	**5**	5 (100.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Marital status													
Single	**46**	29 (63.0)	0.104	2 (4.4)	0.687	8 (17.4)	0.770	3 (6.5)	0.377	1 (2.2)	0.965	3 (6.5)	0.653
Married	**84**	67 (79.8)		1 (1.2)		12 (14.3)		1 (1.2)		1 (1.2)		2 (2.4)	
Cohabiting	**3**	3 (100.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Widowed	**2**	2 (100.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Occupation													
Unemployed	**53**	39 (73.6)	0.773	3 (5.7)	0.093	6 (11.3)	0.251	3 (5.7)	0.285	1 (1.9)	0.945	1 (1.9)	0.096
Formal	**34**	27 (79.4)		0 (0.0)		8 (23.5)		0 (0.0)		1 (2.9)		0 (0.0)	
Informal	**48**	35 (72.9)		0 (0.0)		6 (1.3)		1 (2.1)		1 (2.1)		4 (4.3)	
Specimen													
Nonurine	**37**	25 (67.6)	0.269	1 (2.7)	1.000	8 (21.6)	0.183	1 (2.7)	1.000	1 (2.7)	0.475	0 (0.0)	0.322
Urine	**98**	76 (77.6)		2 (2.0)		12 (12.5)		3 (3.1)		1 (1.0)		5 (5.1)	
Patient status													
Outpatient	**98**	74 (75.5)	0.825	3 (3.1)	0.561	16 (16.3)	0.589	1 (1.0)	0.068	2 (2.0)	1.000	2 (2.0)	0.126
Inpatient	**37**	27 (73.0)		0 (0.0)		4 (14.2)		3 (8.1)		0 (0.0)		3 (8.1)	

be due to the availability of functional genes that directly contribute to pathogenesis or the presence of certain putative factors enabling successful colonization of the host that enhances fitness and adaptation of the bacteria to their surroundings [16] According to Ochman et al. [30], commensal *E. coli* can acquire chromosomal or extrachromosomal virulence operons and become pathogenic.

In furtherance to that, commensal strains may become virulent by a genomic deletion that enhances pathogenicity and random functional point mutations adaptive for pathogenic environments [31]. However, it is unclear whether *E. coli* isolates should be defined as commensals or pathogens based wholly on the source of the specimen and/or phylogenetic group they belong to since phylogroups A and B1 can cause extraintestinal infections in immunocompromised hosts at a point in time [32]. The pathogenicity of *E. coli* isolates belonging to phylogenetic group A is at variance with other studies that categorized pathogenic *E. coli* isolates into phylogenetic groups B2 and D [33, 34]. Our finding is comparable to other studies in other parts of the globe that reported phylogroup A majority [35, 36]. The prevalence of B1 and B2 phylogroups found in the current study are lower than those reported in Abidjan [37]. The three predominant phylogroups, A, B2, and D, reported in our study agree with those stated by Derakhshandeh et al. [38] but at variance with those described by Iranpour et al. [16]. The variations in the distribution of the phylogenetic groups reported in different studies may be due to the health status of the host, geographical and climatic conditions, dietary factors, antibiotic usage, host genetic factors, and the differences arising from different sampling methods [38].

In addition to the four main phylogroups (A, B1, B2, and D) previously reported, the new Clermont quadruplex PCR method of *E. coli* discrimination added four new phylogroups made up of C, E, F, and clade I. In this current study, 4 (3.0%) of the isolates belonged to phylogroup C

Table 4: Association between phylogenetic groups and virulence genes of *E. coli* isolates from clinical specimen.

V. gene	*ChuA*		*P* value	*neuC*		*P* value	*Sfa/foc*		*P* value
Phlyo-group	Present	Absent		Present	Absent		Present	Absent	
A	0 (0.0)	101 (100)		30 (29.7)	71 (70.3)		28 (27.7)	73 (72.3)	
B1	0 (0.0)	3 (100.0)		1 (33.3)	2 (66.7)		0 (0.0)	3 (100.0)	
B2	11 (55.0)	9 (45.0)		6 (30.0)	14 (70.0)		5 (25.0)	15 (75.0)	
C	0 (0.0)	4 (100.0)	**<0.0001**	0 (0.0)	4 (100.0)	0.246	2 (50.0)	2 (50.0)	0.642
Clade I	2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)		0 (0.0)	2 (100.0)	
D	5 (100.0)	0 (0.0)		1 (20.0)	4 (80.0)		2 (40.0)	3 (60.0)	
Total	18 (13.3)	117 (86.7)		40 (29.6)	95 (70.4)		37 (27.4)	98 (72.6)	
V. gene	*fimA*		*P* value	*iutA*		*P* value	*cnfl*		*P* value
Phlyo-group	Present	Absent		Present	Absent		Present	Absent	
A	90 (89.1)	11 (10.9)		69 (68.3)	32 (31.7)		27 (26.7)	74 (73.3)	
B1	3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)		0 (0.0)	3 (100.0)	
B2	16 (80.0)	4 (20.0)		10 (50.0)	10 (50.0)		7 (35.0)	13 (65.0)	
C	4 (100.0)	0 (0.0)	0.6454	3 (75.0)	1 (25.0)	0.403	2 (50.0)	2 (50.0)	0.637
Clade 1	2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)		1 (50.0)	1 (50.0)	
D	5 (100.0)	0 (0.0)		3 (60.0)	2 (40.0)		2 (40.0)	3 (60.0)	
Total	120 (88.9)	15 (11.1)		88 (65.2)	47 (34.8)		39 (28.9)	96 (71.1)	
V. gene	*hlyA*		*P* value	*hlyF*		*P* value	*ibeA*		*P* value
Phlyo-group	Present	Absent		Present	Absent		Present	Absent	
A	30 (29.7)	71 (70.3)		4 (4.0)	97 (96.0)		12 (11.9)	89 (88.1)	
B1	0 (0.0)	3 (100.0)		0 (0.0)	3 (100.0)		0 (0.0)	3 (100.0)	
B2	6 (30.0)	14 (70.0)		3 (15.0)	17 (85.0)		3 (15.0)	17 (85.0)	
C	2 (50.0)	2 (50.0)	0.415	0 (0.0)	4 (100.0)	0.417	0 (0.0)	4 (100.0)	0.468
Clade 1	0 (0.0)	2 (100.0)		0 (0.0)	2 (100.0)		1 (50.0)	1 (50.0)	
D	3 (60.0)	2 (40.0)		0 (0.0)	5 (100.0)		0 (0.0)	5 (100.0)	
Total	41 (30.4)	94 (69.6)		7 (5.2)	128 (94.8)		16 (11.9)	119 (88.1)	
V. gene	*vat*		*P* value	*yfcV*		*P* value	*fyuA*		*P* value
Phlyo-group	Present	Absent		Present	Absent		Present	Absent	
A	42 (41.6)	59 (58.4)		80 (79.2)	21 (20.8)		79 (78.2)	22 (21.8)	
B1	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)		2 (66.7)	1 (33.3)	
B2	9 (45.0)	11 (55.0)		14 (70.0)	6 (30.0)		16 (80.0)	4 (20.0)	
C	0 (0.0)	4 (100.0)	0.322	3 (75.0)	1 (25.0)	0.458	3 (75.0)	1 (25.0)	0.973
Clade 1	2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)		2 (100.0)	0 (0.0)	
D	2 (40.0)	3 (60.0)		4 (80.0)	1 (20.0)		4 (80.0)	1 (20.0)	
Total	56 (41.5)	79 (58.5)		104 (77.0)	31 (23.0)		106 (78.5)	29 (21.5)	

Data are presented as frequencies with corresponding percentages in parenthesis. Phlyo: phylogenetic; V: virulence.

and 2 (1.5%) to clade I. In total, our study reported that 6 (4.4%) of the *E. coli* isolates belonged to the newly described phylogroups against the 13% by Clermont et al. [8] and 25% by Iranpour et al. [16]. This difference in phylogenetic distribution could be due to socioeconomic factors of the study population, climatic conditions, hygienic status, and dietary habits of the host [39].

A significant association was observed with patients' age and phylogenic groups B1 and D in the present study. There are divergent views by various scientists on the impact of age on the phylogenetic distribution of *E. coli* isolates. For instance, Escobar-Páramo et al. [40], in their study, established a link between phylogenicity and patients' age. Their findings were consistent with other studies [41, 42]. On the other hand, a Chinese report did not find any significant difference in the phylogenetic group composition and the age of the patients [43]. The present study did not show any significant difference in the phylogenetic grouping and sociodemographic characteristics of study participants' gender, occupation, marital status, religion, and admission status) except for age. It supports the study by Iranpour et al. [16], who likewise reported no statistical difference in phylogenetic characteristics and patients' gender.

5. Conclusions

In conclusion, this study assessed the relationship between the phylogenetic distribution and the virulence profile of

clinical isolates of *E. coli*. Most of the isolates studied belonged to phylogenetic group A and carried essential factors responsible for virulence and pathogenicity. The virulent genes included adhesin (*fimA*, *yfcv*, and sfa/foc) and haemolysin (*hlyA* and *hlyF*). Patients' age was significantly observed to be associated with phylogenic groups B1 and D. However, there was no association between virulence genes and phylogenetic distribution except *chuA* which was found in all phylogroups D and clade I isolates. The virulence of isolates belonging to phylogroup A, generally considered commensals, is alarming. Therefore, measures must be put in place to control the spread of these virulent strains *E. coli*.

Data Availability

Data is obtainable from the corresponding author upon satisfactory request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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