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Haemolysin and Shigella Toxin Production in Multidrug Resistant *Escherichia Coli* Pathotypes from Clinical Specimens

Iliyasu MY[1]*, Salisu A[1], Mustapha I[2], Shuaibu SM[3], Lawan GM[4], Umar AF[1], Agbo EB[1], Uba A[1] and Deeni YY[5]

[1]Department of Microbiology, Abubakar Tafawa Balewa University, Nigeria
[2]Department of Medical Laboratory Sciences, College of Health Technology, Nigeria
[3]Health Services Department, Abubakar Tafawa Balewa University, Nigeria
[4]Department of Pharmacy Technician, College of Health Technology, Nigeria
[5]Department of Microbiology and Biotechnology, Federal University, Nigeria

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***Corresponding author:** Mahmud Yerima Iliyasu, Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria, Tel: +2348038372210; Email: myiliyasu@atbu.edu.ng

Abstract

The acquisition and dissemination of virulent traits represent a survival advantage to bacterial pathogens. Drug resistance is on the rise among *E. coli* strains that cause human infections. Proper selection of antimicrobial treatment depends on the susceptibility test outcomes. A total of 178 bacterial isolates were phenotypically screened for Haemolysin and Shigatoxin production, then to obtain Multidrug Resistant (MDR) *E. coli*. Twelve isolates were identified and selected based on the ability to grow on Luria-Bertani (LB) agar medium containing 100µg/ml Ampicillin. The isolates, coded as; U01, U02, U03, U04, U08, U10 and U11 were from urine specimens, S05, S06, S07 and S12 from stool, while B09 was from blood. The isolates were screened for multidrug resistant pattern according to Kirby-Bauer disc diffusion method. Genes *hlyA* and *stx1* encoding the virulence factors; Haemolysin A and Shigatoxin1 was PCR amplified and sequenced. All the isolates were resistant to Ampicillin, Cephalothin, Erythromycin, Fusidic acid, Novobiocin and Oxacillin, but sensitive to Colistin sulphate and Imipenem. Nine isolates (75%) are sensitive to Augmentin. All the virulence genes (*hlyA* and *stx1*) are present in isolates S07 and U08. The isolates (75%) produced 2 to 4 of each of the genes indicating a strong relationship in determining multidrug resistance. Haemolysin (*hlyA*) was the most common (66.7%) gene in urine, stool and blood isolates. Most of the virulence genes sequence (61.8%) in this study had significant alignment (95 to 100% homology) with E.coli genome in the NCBI database. This study revealed the interplay of drug resistance and virulence at genetic levels, so advocate for further identification of the mechanisms regulating the expression of these traits, to improve the management of bacterial infections.

Keywords: Multidrug Resistance; Virulence; Haemolysin A; Shigatoxin 1; Colistin Sulphate; Imipenem

Abbreviations: MDR: Multidrug Resistant; LB: Luria-Bertani; CNF: Cytotoxic Necrotizing Factor; Stx: Shigella-Like Toxins; VT1: Verotoxin; EPEC: Enteropathogenic E. Coli; ETEC: Enterotoxigenic E. Coli; EIEC: Enteroinvasive E. Coli; DEC: Diarrhoeagenic E. Coli; Expec: Extraintestinal Pathogenic; UPEC: Uropathogenic *Escherichia Coli*; SMAC: Sorbitol Macconkey; LB: Luria-Bertani; ISA: Iso-Sensitest Agar; BSAC: British Society For Antimicrobial Chemotherapy.

Introduction

Escherichia coli are common non-invasive commensal bacteria established as aetiological agent of various human infections. It is the most prominent cause of infectious diseases that span from the gastrointestinal tract to extra-intestinal sites such as genital and urinary tract infection, septicaemia, and neonatal meningitis [1]. The *E. coli* that cause these diseases have specific pathogenic attributes (virulence factors) that enable them to cause disease [2]. Virulence factors are molecules and structures expressed by microbes like bacteria that enable them to achieve pathogenicity. They are very often responsible for causing disease in the host and converting non-pathogenic bacteria into dangerous pathogens [2]. In bacteria, virulence factors are often encoded on plasmids and chromosomes and can easily be spread through horizontal gene transfer [3].The virulence of the individual strains in a given infection is determined by the presence and the actual expression of the virulence genes which are found in the organisms, as well as by the environmental conditions in the host [4].

The virulent factors of *E.coli* play a vital role in the process of pathopoiesis. Pathogenicity of *E. coli* is due to the presence of many virulence genes that encodes some important virulence factors, such as fimbriae (fimH), invasive plasmid adhesin (ipaH),intimin (eaeA), cytotoxic necrotizing factor (CNF), haemolysin (hlyA), bundle forming pili (bfpA), heat-labile toxin (*elt*), heat-stable toxin (est), Shigella-like toxins (stx) and verotoxin (VT1) [5]. Several virulence genes have been used to detect human diarrhoeagenic *E. coli*. For example, *eaeA, est and elt, and ipaH* are typically found in enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and enteroinvasive *E. coli* (EIEC), respectively, and have been used as genetic markers to detect their corresponding pathotypes [6,7]. Diarrhoeagenic *E. coli* (DEC) cause infection by a variety of complex mechanism, which include adherence toxins, elaboration of toxigenic mediators, and invasion of intestinal mucosa and transportation of bacterial proteins into the host cells [8]. Adherence to solid surfaces is a common characteristic of many extraintestinal pathogenic (ExPEC) bacteria [9]. By attaching to host structures, microbial pathogens avoid being swept along by the normal flow of body fluids (blood, urine, intestinal contents) and eliminated [9]. Uropathogenic *Escherichia coli* (UPEC) are the most common bacteria causing UTIs [10]. They possess specialized virulence factors, enabling them to colonize and invade to the host, disrupt the host defence mechanisms, injure host tissues, and/or stimulate a noxious host inflammatory response [11]. Virulence factors of recognized importance in the pathogenesis of UTI include diverse adhesins, toxins, siderophores, and polysaccharide capsules [10].

Bacterial virulence is responsible for rapid dissemination of these pathogens, which results in avoidable therapeutic failures in patients on antibiotics [11] and outbreaks of multidrug resistant pathogens that required expensive drugs. The treatment of *E. coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by the virulent strains [6]. Identification of intestinal *E. coli* strains requires that these organisms be differentiated from non-pathogenic members of the normal flora. Sero-grouping of O antigen is not sensitive-enough to identify a strain as diarrhoeagenic, as it does not mostly correlate with the presence of virulence factors [9]. Thus, identification of diarrhoeagenic *E. coli* strains needs to detect genes that determine the virulence of these organisms. With the advent of PCR and gene sequencing analysis, it has become possible to detect ESBLs and virulence genes in bacterial isolates, allowing the rapid diagnosis of infections caused by pathogenic *E. coli*.

Previous studies describing the clinical significance of virulent *E. coli* infections have focused mainly on phenotypic characterization of isolates from cases of UTIs [11]. Measuring a phenotype *in vitro* does not always correlate with *in vivo* expression and may underestimate the presence of a virulence factor in a particular strain [10]. Phenotypic methods cannot differentiate between the specific genes responsible for pathogenicity. Molecular studies of pathogenic bacteria hold significant promise in understanding the virulence factors thereby identifying coordinates highly relevant in the development of treatment strategies. The aim of this study is therefore, to determine the genotypic characteristics of virulent *E. coli* pathotypes isolated from clinical specimens.

Materials and Methods

Bacterial Isolates

A total of 178 *Escherichia coli* isolates were screened on 5% sheep blood agar for haemolysin production and inoculated onto Sorbitol MacConkey (SMAC) agar media for phenotypic identification of suspected Shigatoxin producing *E. coli* (STEC) colonies [12]. The isolates were also selected based on the ability to grow on Luria-Bertani (LB) agar medium containing 100µg/ml Ampicillin. Twelve isolates phenotypically confirmed for these virulence factors (Haemolysin and Shigatoxin) were used for genotypic characterization.

Screening of Isolates for Multidrug Resistance Characteristics

The isolates were screened for antimicrobial

susceptibility pattern, multidrug resistant characteristics on Iso-sensitest agar (ISA) media (Oxoid, UK) according to Kirby-Bauer disc diffusion methods as described by Mahon C, et al. [13]. The following commercial discs were used:Combined discs M13/M14 rings (Mast Diagnostics, UK): Chloramphenicol (5µg), Erythromycin (5µg), and Fusidic acid (10µg), Oxacillin (5µg), Novobiocin (5ugµg), Streptomycin (10µg), Tetracycline (25µg), and M14 rings: Amoxycillin (30 µg), Cephalothin (5µg), Colistin sulphate (25µg), Gentamycin (10µg), Sulphatriad (200µg), and Cotrimoxazole (25 µg). Single discs (Oxoid): Amoxycillin-Clavulanate/Augmentin (30µg), Cefuroxime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ciprofloxacin (10µg) and Imipenem (10µg). The diameter zone of inhibition was measured in millimetre and results were interpreted according to British society for antimicrobial chemotherapy (BSAC) guidelines [14]. *Escherichia coli* ATCC 35401 were used as positive control; while *E. coli* K-12 DH5α as negative control.

Detection of Virulence Genes

DNA Extraction

The clinical isolates were grown for 24 hours on Luria-Bertani (LB) agar plates (containing 100µg/ml ampicillin). Chromosomal DNA was isolated from overnight bacterial culture by boiling method as described by British Society for Antimicrobial Chemotherapy [15]. A loopful of cells from a single colony was transferred to 100µl of sterile/double distilled water and the mixture was boiled for 10 min, at 95°C in water bath to lyse the cells. The cell lysate were then centrifuged briefly (10s at 10,000 rpm). The supernatant was carefully pipetted into fresh tube and kept at -20°C for further use. A 5µl of the sample was used for the PCR reaction [16]. Plasmid DNA was isolated from the samples using Qiagen miniprep protocol by alkaline lysis method according to manufacturers' instructions.

PCR Amplification of Virulence Genes

Virulence genes were amplified using *hlyA* and *Stx1* specific primers (Integrated DNA Technologies, USA). PCR was performed in a final reaction volume of 25 µl, which comprises of 5.5 µl sterile distilled water, 1 µl (each) reverse and forward primers, 12.5 µl of Master mix, Taq DNA polymerase, and 5 µl of bacterial lysate (supernatant with template DNA). Amplifications were performed with the G-Storm thermocycler GS0001 (ThermoFisher Scientific, UK). PCR amplification start with initial denaturation at 94°C for 5 minutes, then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 1 minute. A final extension at 72°C for 10 minutes was conducted, as described by Li, et al. [7]. A molecular marker 1 kb DNA Hyperladder (Bioline, UK) was used to assess the PCR product size (Table 1).

Gene	Primer	Oligonucleotide sequence (5′- 3′)	Size (bp)	Reference
HlyA	HlyA - F	AACAAGGATAAGCACTGTTCTGGCT	1177	Jalali, et al. [17]
	HlyA – R	ACCATATAAGCGGTCATTCCCGTCA		
Stx1	Stx1-F	CAGTTAATGTGGTGGCGAAGG	348	Rono, et al. [18]
	Stx1-R	CACCAGACAATGTAACCGCTG		

Table 1: Characteristics of primer for amplification of virulence genes in the bacterial isolates.

The product was separated by gel electrophoresis on 1.0 % agarose, stained with gel red and the image was captured digitally with UV transillumination (Syngene).The product size was estimated using 1kb DNA Ladder (Bioline, UK). The PCR products was purified and stored at 4°C, before sequencing. The characteristics of the primers used and the expected amplicon size are given as shown above.

Sequencing Analysis for Virulence Genes Identification

The purified DNA from the PCR products was finally analyzed by sequencing techniques for the genes identification at the Medical Research Institute, University of Dundee (Scotland), UK.

Statistical Analysis

The data of the study was analyzed by using the SPSS version 21.1. Chi-square test was used to compare the difference in virulence genotypes in relation to MDR profile. Probability level (P<0.05) was considered statistically significant.

Results and Discussion

Multidrug Resistant Characteristics of the Isolates

Multidrug resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single

antibiotic resistance phenotype. It has been shown that antibiotics therapy can select for antibiotic resistant strains in the enterobacterial flora and plasmid-mediated antibiotic resistance can spread in a population subjected to heavy antibiotic therapy [19]. In the present study, (Table 2) all the twelve isolates (100%) are resistant to Amoxycillin, Cephalothin, Erythromycin, Fusidic acid, Novobiocin and Oxacillin. Eleven isolates (91.7%) are resistant to Chloramphenicol, Cotrimoxazole, Streptomycin and Tetracycline. Eight isolates (66.7%) are resistant to Ciprofloxacin. Seven isolates (58.3%) are resistant to Cefotaxime, Ceftriaxone Cefuroxime and Gentamycin. Nine (9) isolates (75%) are sensitive and three (3) isolates (25%) are resistant to Augmentin. However, the isolates were sensitive to Colistin sulphate (100%), Imipenem (100%), and Augmentin (75.0%).The *E.coli* isolates U03, U04, S05, S06, S07, B09, U10, U11 and S12 were sensitive to Augmentin and at least five of the isolates are also sensitive to Cefotaxime, Ceftriaxone, Cefuroxime and Gentamycin. The result showed higher resistance of the isolates to beta-lactams like ampicillin which alarms us that such drugs should no longer be used as first line of treatments in this area, as it used to be in the previous decades. These results of ampicillin-resistant *E.coli* are congruent to the report of Farshad, et al. [20], who found 100% resistance of their *E.coli* isolates to ampicillin.

Antibiotics (µg)	Number of isolates (n=12) and Susceptibility pattern (%)	
	No. (%)Sensitive	No. (%) Resistant
Amoxycillin (30)	00(0.0)	12(100)
Augmentin (30)	03(25.0)	10(75.0)
Ceftriaxone (30)	05(41.7)	07(58.3)
Cefuroxime (30)	05(41.7)	07(58.3)
Cefotaxime (30)	05(41.7)	07(58.3)
Cephalothin (5)	00(0.0)	12(100)
Ciprofloxacin (5)	04(33.3)	08(66.7)
Cotrimoxazole (25)	01(8.33)	11(91.7)
Colistin sulphate (25)	12(100)	00(0.0)
Erythromycin (25)	00(0.0)	12(100)
Fusidic acid (5)	00(0.0)	12(100)
Gentamycin (10)	05(41.7)	07(58.3)
Imipenem (10)	12(100)	00(0.0)
Novobiocin (5)	00(0.0)	12(100)
Oxacillin (5)	00(0.0)	12(100)
Tetracycline (30)	01(8.33)	11(91.7)

Table 2: Distribution of bacterial isolates according to Antibiotic susceptibility pattern.
N.B: Zones of inhibition was interpreted according to BSAC, guidelines (2013)

The greater prevalence of resistance to common antibiotics was reported by Mohajeri, et al. [10] where maximum number of isolates (100%) was resistant to ampicillin, carbenicillin and ceftazidime and the lowest to chloramphenicol (37%). A maximum resistance among *E. coli* species isolates was observed against cefpodoxime 100% by Sahu, et al. [21]. The presence of multidrug resistance in this study may be related to the dissemination of antibiotic resistance among hospital isolates. Zhao, et al. [22] observed that *Escherichia coli* are one of the main bacterial pathogens responsible for nosocomial infections especially in immunocompromised patients. A high sensitivity of *E. coli* strains to Imipenem has been previously reported by Farshad, et al. [20]. It seems this antibiotic can serve as a medication of choice for the treatment of UTI caused by *E. coli*. However, it should be noted that unlimited use of a chemotherapeutic agents can gradually lead to rising antibiotic resistance.

Genotypic Characteristics of Virulence Factors

The ability of some bacteria like *E. coli* to cause diseases is determined by harbouring and acquisition of some pathogenic attributes [23]. For example, presence of cytotoxins and haemolysins in these organisms is responsible

for established UTI in some women and diarrhoeal cases of medical importance [2]. In the present study (Figures 1 & 2), all the virulence genes, hlyA and stx1 produced were found in isolates S07 and U08. This is followed by U01, U02, S06 and B09 which produced 3 of the genes, while U10 and S12 are the least with only one gene each. The results of this study show that virulence genes are more commonly found among gastroiontestinal bacteria (stool isolates) than in other cases such as UTI.

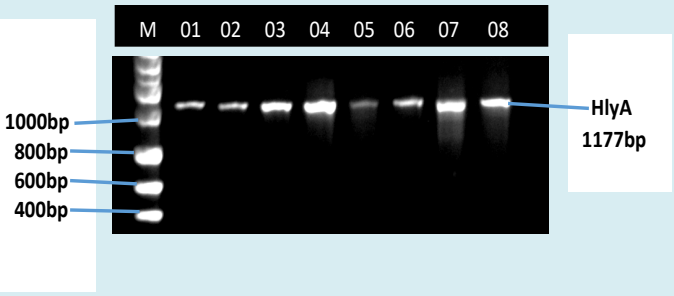

Figure1: Haemolysin A gene PCR products (chromosomal DNA) agarose gel image. Lane 01, 02, 03, 04, 05, 06, 07 and 08, are isolates that harbours hlyA gene with expected amplicon size of 1177bp [17]. Lane M is molecular marker (1kb DNA ladder, Bioline, UK).

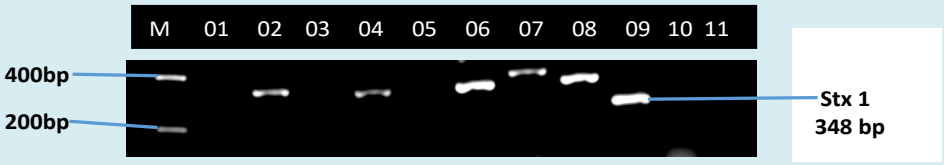

Figure 2: Shiga toxin 1 gene PCR products (chromosomal DNA) agarose gel image. Lane 02, 04, 06, 07, 08 and 09, are isolates that harbours stx11 gene with expected amplicon size of 348 bp [18]. Lane M is molecular marker (1kb DNA ladder, Bioline, UK). Lane C is a control strain, ATCC 3450 (Virulent E. coli).

The above figure shows that the isolates, U01, U03, S05, S06, S07, U08, B09, U10, U11 and S12, harbours stx1 gene.

N.B: Zones of inhibition was interpreted according to BSAC, guidelines (2013) Birosova, et al. [23] reported that alpha-haemolytic *E. coli* strains are probably found more frequently in the intestinal tract than urinary tract. But in previous studies Tarchouna, et al. [24] reported frequent occurrence of fimH and haemolysin in patientwith UTI. Haemolysin and adherence through fimbriae are important properties of the uropathogenic *E.coli*. The degree of severity depends on the virulence of the responsible strains and on the susceptibility of the host [25]. A better cognition of the virulence characteristics of the microorganisms causing infection will permit the clinicians to anticipate the evolution of infection in the host. Many virulence determinants contribute to the pathogenicity of *Escherichia coli*.

The results indicated that the urine isolates with this high prevalence of virulence factors can be a major causative agent for UTIs in humans in the study area. In spite of the previous studies, our results showed that the urine isolates have a different virulence profile. These differences in prevalence of virulence genes showed that the properties is closely depending on geographic region and even weather conditions of each regions. It seems that the epidemiology and prevalence of virulence factors of urine isolates from patients with UTI is different in this area. It was also confirmed that haemolysin is important in pathogenesis and the pathogenicity of these gene encoding isolates. It also depends greatly on the ability of the gene to switch between conformations and this is dependent on the different alleles that can be expressed by this gene [26- 28]. Another important secreted virulence factor of uropathogenic *E. coli* also found in our isolates is a lipoprotein, α-haemolysin (HlyA), which is associated with upper UTIs such as pyelonephritis. Haemolysin production is associated with pathogenicity of *E.coli*, especially in the more severe forms of infection [22] (Table 3).

Virulence genes	Specimens (n=12)/Source of Isolates			
	Blood (n = 01)	Stool (n=04)	Urine (n=07)	Total(%) positive
HlyA	01	02	05	08 (66.7)
Stx 1	01	02	03	06 (50.0)

Table 3: Genotypes of Virulence factors according to type of specimen.

At high concentrations, HlyA is able to lyse erythrocytes and nucleated host cells, a process that may enable extraintestinal pathogens like UPEC to better cross mucosal barriers, damage effect or immune cells, and gain enhanced access to host nutrients and iron stores [29]. Haemolysin is known to confer selective advantage to the pathogen by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic cells and epithelial cells. Haemolysin production has also been shown previously to influence pathogenicity. Haemolysins has been proposed to inflict direct cytotoxic effects on renal epithelium [22,26].

The hlyA gene prevalence (66.7%) in our studies is significantly associated ($p<0.05$) with both UTI and gastrointestinal disorders, as the gene was detected in both urine and stool isolates. There was a clear relation between tissue damage and the presence of haemolysin in this study. Prevalence of these genes differs on the basis of phylogenetics, geographical distribution, and clinical presentation. A huge variation in the frequencies of these genes was recorded worldwide [24]. Haemolytic activity in *E. coli* has been attributed to many types of Haemolysin genes. A new class of haemolysin represents a novel bacterial gene designated *hlyF* gene that is strongly associated with *E. coli* isolates from bloodstream infection [30].

Virulence is due to a plasmid such as *HlyA* that encodes genes required for invasion, cell survival and apoptosis of macrophages [10]. The virulence genes in *E. coli* are often located on transmissible genetic elements that can be transferred to *E. coli* recipient strains**.** These virulence determinants give each pathotype the capacity to cause a clinical syndrome with distinctive epidemiologic and pathologic characteristics [27] and are therefore ideal targets for the determination of the pathogenic potential of any given *E. coli* variant. Shiga toxin (stx) is one of the major virulence factors involved in *E. coli* O157:H7 pathogenesis based on immune reactivity, toxins are classified as either Stx1 or Stx2, which damage intestinal epithelial cells and kidneys, causing haemorrhagic colitis and haemolytic uremic syndrome, respectively [9]. The stx gene in *E. coli* O157:H7 is associated with a prophage, and different subtypes of shiga-toxin are identified [24].

The prevalence of stx genes in our studies was low. However, these virulence genes are usually associated with occurrence of diseases. A study in Egypt by Morsi and Elsaid Tash [31], showed similar distribution of virulence genes. This indicates that some social and environmental factors may contribute in the virulence pattern of *E. coli* in different communities. The negative isolates for some of the genes in our study may be part of the normal flora that lack these virulent genes or may be due to the possibility of corresponding gene mutations, as negative test results does not always indicate the absence of the corresponding operon while a positive result usually confirms the presence of the virulence genes. When antibiotic resistance was tested among the virulent isolates, a significant relation was observed ($P<0.06$), where most of the isolates harbouring the virulence genes were multidrug resistant.

Shigella toxin-producing *E. coli* (STEC) were identified as the cause of diseases in human host due to its association with *E. coli* O157:H7 involved in diarrhoea, purpura and the haemolytic uremic syndrome. Many water and food-borne disease episodes have been reported from different geographical areas worldwide [30-33]. Whatever, STEC is associated with a wide range of human diseases such as bloody diarrhoea, haemorrhagic colitis (HC), and haemolytic-uremic syndrome (HUS). The Shiga toxins either 1 (*stx1*) or 2 (*stx2*) interfere the binding of aminoacyl tRNA to the ribosomes and preventing the protein synthesis resulting in depurinating specific residues of the host cell ribosomes after internalization. The biological activities of *stx1* and *stx2*, involving cytotoxicity to Vero and HeLa cells, are similar, but the immunological properties are different [33].

Virulence Gene Sequences with Significant Alignment

Escherichia coli are a common and widespread bacterium associated with various infectious diseases of both intestinal and extraintestinal sites. This is due to acquisition and dissemination virulence genes that determined their pathogenicity. The worldwide burden of these diseases is staggering, with hundreds of millions of people affected annually. Eight *E. coli* pathovars have been well characterized, and each uses a large arsenal of virulence factors to subvert host cellular functions to potentiate its virulence [33,34]. Haemolysin A (hlyA) gene from isolates S12 shows a high significant sequence alignment at 97% homology with the EPEC strain, *E. coli* O128:H27 chromosomal DNA, complete

genome (CP024243.1). Enteropathogenic *Escherichia coli* (EPEC) strains were the first *E. coli* strains recognized as important pathogens in diarrhoeal diseases [6]. Even today, EPEC strains are the major bacterial cause of neonatal and infantile gastroenteritis throughout the world, especially in developing countries [33]. Enteropathogenic *Escherichia coli* (EPEC) implicated in gastrointestinal infections represents a major causative agent of infant diarrhoea [35]. It has been recognized to exhibit a great pathogen-attributable risk of death in infants eleven months of age and younger [36]. Studies have shown that its morbidity and mortality rates, especially in developing countries, are quite significant [37].

It is conceivable that virulence genetic determinants, if located on the same genetic platform as antimicrobial resistance genes, may be co-mobilized under antimicrobial selective pressure [38]. In this study, isolates S07 and U08 which carry all the virulence genes are resistant to 17 (85%) out of the 20 antibiotics tested, while U01 and S06, which harboured 3 of the virulence genes are resistant to 18 (90%), with U02 (85%) and B09 (65%). The remaining isolates which mainly carry hlyA genes are resistant to 10 to 17 antibiotics. The control of *E. coli* infections is therefore becoming complicated due to the increasing resistance to antibiotics (Table 4).

DNA Sample	Identified strain	Gene	Type of genome	Sequence ID	% ID
HlyA 03	*E. coli* EC11	cDNA	complete genome	CP027255.1	97
HlyA 05	*E. coli* O128:H27	cDNA	complete genome	CP024243.1	97
HlyA 06	*Ent. hormaechei* 34983	cDNA	complete genome	CP010377.1	94
HlyA 08	*K. pneumoniae* KpvK54	cDNA	complete genome	CP023134.2	84
HlyA 09	*E. coli* strain MEM	cDNA	complete genome	CP012378.1	93
HlyA 10	*E. coli* O104:H4	cDNA	complete genome	CP027394.1	97
HlyA 11	*E. coli* RM14721	cDNA	complete genome	CP027105.1	98
HlyA 12	*E. coli* HS13-1	cDNA	complete genome	CP026491.1	91
Stx1 06	*Ent. cloacae* AR-0093	cDNA	complete genome	CP027604.1	95
Stx1 07	*P. aeruginosa* AR-357	cDNA	complete genome	CP027166.1	93
Stx1 08	*K. pneum.*WCHKP649	cDNA	complete genome	CP026585.2	100
Stx1 09	*E. coli* RM14721	cDNA	complete genome	CP027105.1	100
Stx1 12	*E. coli* WCHEC05237	cDNA	complete genome	CP026580.1	100

Table 4: Virulence genes sequence producing significant alignment.

Conclusion

This study observed that the virulence genes hlyA and stx are harboured in the isolates,indicating a strong relationship in determining MDR of *E.coli* pathotypes. Therefore increased alertness of clinicians and enhanced testing of virulence factors by laboratories are necessary to reduce chemotherapeutic failure. The present study has shown the capacity of *E. coli* to adapt and survive in different tissues through virulence and developing drug resistance. The expression of virulence factor(s) may depend on the pathogens and varies in different kinds of infections. Most of the isolates in this study are resistant to commonly used antibiotics, but continued prescription of Augmentin is still advocated.

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