

Microbial Science Archives (ISSN: 2583-1666)

An International Research Journal

Journal homepage: www.microbial.sciencearchives.org

<https://doi.org/10.47587/MSA.2023.3108>



Research Article

Prevalence of virulent, pathogenic and colistin resistant genes in *Escherichia coli* isolated from out-patients attending Faith Mediplex, Benin City, Edo state, Nigeria

Elma Aniekan Lisk-Anani¹, Martin Duru², Lillian Olere Inegbedion² and Iyekhoetin Matthew Omoruyi¹  

¹Department of Biological Sciences, Faculty of Science, Benson Idahosa University, P.M.B. 1100, Benin City, Edo State, Nigeria

²Laboratory Department, Faith Mediplex Hospital, No. 1 Giwa Amu/Airport Road, GRA, Benin City, Edo State, Nigeria

Received: Feb 10, 2023/ Revised: Mar 5, 2023/Accepted: Mar 17, 2023

(✉) Corresponding Author: iomoruyi@biu.edu.ng

Abstract

Multidrug resistance is emerging as a serious threat to public health causing increase mortality globally. This study was aimed at investigating the prevalence of multidrug resistant *Escherichia coli* possessing *mcr-1*, *Uida* and *Lt* genes isolated from out-patients attending Faith Mediplex, in Benin City. A total of 18 *E. coli* isolates were collected over a period of three months from two sources (urine and high vaginal swab). Samples were obtained using sterile universal collection bottles and swab sticks and cultured using the streak plate method on Eosin Methylene Blue (EMB) agar. The isolates were further identified by their morphological, biochemical as well as 16S rRNA analysis. DNA extraction was done by boiling method, while the presence and/or absence of colistin resistance and virulence genes were determined by polymerase chain reaction and gel electrophoresis. Antibiotics susceptibility profile of the isolates was determined by the disc diffusion method. Most of the *E. coli* species isolated were resistant to nalidixic acid, amoxicillin clavulanate, ampiclox, oxacillin and levofloxacin, all isolates were highly susceptible to colistin and ceftriaxone sulbactam. All except one organism was resistant to nitrofurantion. Multidrug resistance was observed in most of the isolates and the multidrug resistance index ranged from 50.0% to 71.4%. Four out of the 18 isolates had the presence of beta-glucuronidase (*uidA*) gene and only 2 out of 18 isolates had the heat-labile (*Lt*) gene. The other genes were not present in all 18 isolates. The presence of this isolate in clinical samples constitute serious public health concern hence individuals should be educated on personal hygiene and environmental sanitation, antimicrobial stewardship be incorporated in hospitals to reduce multidrug resistance and constant testing should be done because of the life-threatening diseases this organism causes.

Keywords: Virulence gene; *Escherichia coli*; Public Health; Hospital; Antimicrobial stewardship.

Introduction

Antimicrobial resistance (AMR) is one of the major concerns of this century, which has made the effective treatment of infectious diseases very challenging. Microorganisms can use several mechanisms such as genetic alterations, mutation etc. to adapt to antimicrobial stimulants (Munita and Arias, 2016).

Colistin, also known as polymyxin E, is a polypeptide antibiotic that belongs to the class of polymyxin antibiotics produced by *Paenibacillus polymyxa* (Ahmed et al., 2020). Colistin was first discovered in 1947, and was immediately recommended for use in human medicine (Giske, 2015). However, it was abandoned in the mid-1980s, due to its neurotoxic (toxicity affecting the central nervous system or

brain) and nephrotoxic (toxicity in the kidneys) side effects (Giske, 2015). As an old class of antimicrobials, it has been increasingly used in recent decades as a last resort drug to treat infections caused by multi-drug resistant (MDR) and the carbapenems-resistant bacteria in human medicine (Giske, 2015).

Colistin targets the outer membrane lipopolysaccharide (LPS) of Gram-negative bacteria. Binding of colistin to LPS increases the permeability of bacterial membrane and leads to leakage of the contents in the cytoplasm and ultimately cell death (Ahmed et al., 2020), with its primary mechanism of resistance being the modification of lipid A of the LPS (Liu et al., 2016). In China in the year 2015, colistin-mediated resistance gene (*mcr-1*) was identified. It encoded an enzyme that catalyzes the addition of phosphorylethanolamine to the phosphate groups in lipid A that was found in a conjugative plasmid in *Escherichia coli* from animal origin (Liu et al., 2016). Since then, numerous studies have reported plasmid-borne *mcr-1* to 8 in several colistin resistant Gram negative bacteria (Yin et al., 2017; Wang et al., 2018; Gelbicova et al., 2019; Carroll et al., 2019; Borowiak et al., 2019).

Among all Enterobacteriaceae, colistin resistance has been studied extensively in *Salmonella* and *Klebsiella pneumoniae* because their regulatory genes (*mgrB*, *phoPQ*, and *pmrAB*) are important mechanisms to their resistance (Falagas et al., 2010; Cannatelli et al., 2013). However, in *E. coli*, the mutations in *mgrB* and *phoPQ* have not been reported to cause colistin resistance (Falagas et al., 2010). Colistin resistance in clinical *E. coli* strains is caused by mutations in the *basRS* gene (Cannatelli et al., 2017), although experimental validation of the role of these mutations is currently lacking. Research carried out by Marchaim et al. (2011), at Detroit Medical Center reported cases of colistin resistant carbapenem-resistant *Klebsiella pneumoniae* in the United States which occurred occasionally but not as part of an outbreak. The main mechanism of transmission was due to patient-to-patient transmission and not the emergence of resistance (Marchaim et al., 2011). According to their study, the hospital where all the patients resided was where the most transmissions occurred. The outbreak was stopped when strict infection, control practices were implemented (Marchaim et al., 2011). Other than the acquisition of the *mcr* gene by *E. coli* other mechanisms of colistin resistance is unknown (Luo et al., 2017). According to research data provided by Dadashi et al. (2021), the prevalence rate of colistin resistant *E. coli* in clinical samples were higher in Cambodia, Malaysia, Saudi Arabia and Oman by 100% (Dadashi et al., 2021). In Africa, overall resistant to *E. coli* isolated from clinical samples were at 1.25% and the highest report was in Algeria by 100% (Dadashi et al., 2021). After that, the highest reported cases were in Nigeria and Burkina Faso by 38.36% and 61.29%, the rate of prevalence of *E. coli* was recorded the lowest in Tunisia by 0.15% (Dadashi et al., 2021). The risk of transmission is increased because animal and human gut colonizers can exchange genetic material with other bacteria, including both commensal and pathogenic bacteria (Madni et

al., 2022). The *mcr* genes can subsequently be passed on to progeny (offspring) cells through vertical transmission (generational transmission of viruses from parent to offspring). Some studies have shown that the *mcr* genes can be found in various species, including *E. coli*, *Klebsiella pneumoniae* (Zhang et al., 2019), *Salmonella enterica* (Borowiak et al., 2019), and *Enterobacter* species (Chavda et al., 2019) from animals, food products, human sources and the environment. Most of the *mcr*-harboring isolates are Enterobacteriaceae, which are common to both animals and humans.

The aim of this study was to investigate the prevalence of multidrug resistance and virulence gene in *Escherichia coli* isolated from out-patients attending Faith Mediplex, Benin City, Edo State, Nigeria.

Methods and Materials

Sample collection

Clinical samples were gotten from Faith Mediplex, Benin City, Edo State, Nigeria, between March and May, 2022.

Isolation of *Escherichia coli* from out-patients

The isolation and identification of *Escherichia coli* from the clinical samples was done by streaking swab of already cultured suspected *E. coli* gotten from Faith Mediplex. The isolate was cultured on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hrs. After 24 hrs of incubation, the isolates were observed to have green metallic sheen and biochemical tests were carried out to identify the organism further.

Preservation of bacterial isolates

The isolates gotten from each culture was preserved on agar slant. The agar slant was prepared using double strength of Nutrient agar, and the organism was inoculated on the surface of the agar and incubated for 24-48 hrs at 37°C and refrigerated for subsequent use.

Identification of *Escherichia coli*

Escherichia coli was identified by their cultural, morphological, biochemical and 16S rRNA analysis. The biochemical test included Gram reaction, catalase, citrate, hydrogen sulphide production, indole, Mannitol, lactose, fructose and glucose fermentation.

Antibiotics susceptibility test

All *Escherichia coli* species isolated were tested for their resistance and/or sensitivity to the following antibiotics: amoxicillin-clavulanate (30µg), cefotaxime (25µg), imipenem/cilastatin (10µg), ofloxacin (5µg), gentamycin (10µg), nalidixic acid (30µg), nitrofurantoin (300µg),

cefuroxime (30µg), ceftriaxone sulbactam (45µg), ampiclox (10µg), cefexime (5µg), levofloxacin (5µg).

The antibiotic susceptibility pattern was determined according to the procedures described by the National Committee for Clinical Laboratory Standards (CLSI, 2020). The bacterial isolates were inoculated in peptone water and incubated at 37°C for 24hrs. Turbidity of the broth was adjusted with sterilized peptone water to get a density of 0.05-0.1 McFarland turbidity standard with the use of a spectrophotometer. Sterile swab sticks were used to streak the broth onto prepared Mueller Hinton agar plates. Sterile forceps were used to pick and place the antibiotic disc on the surface of the inoculated media. The plates were inverted and incubated at 37°C for 24hrs. After 24hrs of incubation, zones of inhibition appeared and the results were read using a millimeter ruler to measure each zone.

Genomic dna extraction (boiling method)

The technique employed was the boiling method; the isolates from stored slant were sub cultured unto nutrient agar and incubated at 37°C for 18 hrs. The fresh isolates were put into 2ml Eppendorf tubes containing 200µl of distilled water using sterile inoculating loop after which each suspension was vortexed for 2 min at maximum speed using a vortex shaker. The suspension was then centrifuged for 10 min at 13000 rpm (revolution per minute) to break the cells. After centrifugation, 500ml of distilled water was added to the Eppendorf tubes and vortexed for another 2 min. The mixture was placed into the water bath at 100°C for 15 min to finally release the DNA from the cells. The DNA was then harvested by centrifugation at 10,000 rpm for 5 min and collected from the supernatant and store at -20°C in sterile Eppendorf tubes for future use.

Detection of virulence gene in *Escherichia coli* using polymerase chain reaction

Polymerase Chain Reaction (PCR) was done to amplify target genes in the template DNA using forward and reverse primers specific to each gene (*uidA*, *fliCH7*, *lt*, *stx*, *mcr-1* to *mcr-8*) as previously described (Omoruyi and Ojubiaja, 2022; Omoruyi et al., 2023). Each PCR tube contained 12.5µl of Taq quick load Master Mix, 0.5µl of the forward and reverse primers, 1.5µl of the template DNA and 10µl of nuclease-free water. The tubes were placed inside the thermocycler and the machine was set with the appropriate cycles and temperatures as described by Omoruyi and Ojubiaja (2022).

Gel electrophoresis

After the PCR reaction was completed, the next step was gel electrophoresis. 2g of agar powder was weighed and dissolved in 100mL of 1x TAE (Tris – Acetate EDTA) buffer and heated to boiling to completely dissolve it. After dissolving, it was left to cool for some minutes and 2.5µl of Ethidium bromide was added to the gel. The gel was poured into the gel trays which have combs in it. The combs help to form wells in the

gel. Then it was allowed to solidify. When it had completely solidified, the combs were removed. The gel in the tray was put into the electrophoresis machine and 1x TAE buffer was also poured into the machine. 2.5µl of the loading dye was added to the samples that were brought out of the PCR machine and mixed together. 10µl of the molecular weight marker was put into the first well of the first gel, and the same 10µl into the first well of the second gel. 10µl of all samples were carefully put it into the wells. The machine was covered and put on. It was allowed to run for 45min at 100V. After 45min the machine was put off and the gel tray taken out. The gel was taken out of the tray and placed on the UV transilluminator. The UV light shows the bands of the DNA.

Polymerase chain reaction (PCR) and 16S rRNA analysis

PCR sequencing preparation cocktail consisting of 10µl of 5x Go Taq colourless reaction, 3µl of 25mM MgCl₂, 1µl of 10mM of dNTPs mix, 1µl of 10pmol each of the desired gene. The PCRs was conducted using universal primers, in a Gene Amp 9700 PCR System Thermal cycler (Applied Bio system Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30sec, 30sec annealing of primer at 56°C and 72°C for 1min 30sec; and a final termination at 72°C for 10min and hold at 4°C.

Blast analysis

The blast analysis was done on the National Centre for Biotechnology Information (NCBI) website (<http://blast.ncbi.nlm.nih.gov/>). DNA sequences of each of the test organism was copied in fasta format into the nucleotide sequence search engine and used to query the NCBI data base in search of sequences producing significant alignments with a view to determining the best fit identity for each of the test organisms. The 16S rRNA partial sequence of the test isolates were submitted to GenBank (NCBI) with receipt of corresponding GenBank accession numbers.

Results

Table 1. shows the prevalence of *Escherichia coli* isolated from patients attending Faith Mediplex Hospital. *Escherichia coli* was isolated in all 18 samples between March and May 2022. All samples were obtained from urine and high vaginal swab. A total of 18 isolates were obtained and screened based on their cultural, morphological and biochemical characteristics (Table 2) as well as by 16S rRNA analysis, which confirmed the isolate to be *Escherichia coli* O157:H7 (Table 3). The phylogenetic tree of evolutionary relatedness also shows that *Escherichia coli* O157:H7 was the most closely related isolate to the sequence obtained, when compared with *Escherichia coli* strain K-12, *Escherichia marmotae*, *Shigella boydii* etc (Fig. 1).

The antibiotic susceptibility pattern of *Escherichia coli* isolated from patients at Faith Mediplex against 12 antibiotics

is reported in Table 4. According to the results, isolate FM2 was susceptible to all the antibiotics used. All isolates apart from FM2 were resistant to nalidixic acid (NA). Also, all isolates apart from FM11 were susceptible to nitrofurantoin (NF). FM18 was susceptible to all antibiotics apart from nalidixic acid. The mean percentage sensitivity, intermediate and resistant was 45.34%, 6.92% and 47.64% respectively (Table 4). FM1, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15 and 16 were observed to be multidrug resistant (Table 5). There was no phenotypic resistance to colistin amongst the isolates, while the majority (10 isolates) was resistant to methicillin (Table 6). Of the 18 isolates, only 4 (FM5, FM12, FM16 and FM18) had the *uidA* gene, 2 (FM8 and FM16) had the *Lt* gene, while none had the *stx* and *fliCH7* gene. Only FM16 had both *uidA* and *Lt* genes (Table 7).

Table 1. Prevalence of *Escherichia coli* Isolated from Patients at Faith Mediplex Hospital

Isolates Code	Date of collection	Sample source	<i>E. coli</i>
FM 1	17-03-2022	Urine	Present
FM 2	17-03-2022	Urine	Present
FM 6	20-04-2022	Urine	Present
FM 7	20-04-2022	HVS	Present
FM 8	20-04-2022	Urine	Present
FM 9	20-04-2022	Urine	Present
FM 10	20-04-2022	HVS	Present
FM 11	20-04-2022	Urine	Present
FM 12	20-04-2022	Urine	Present
FM 15	20-04-2022	HVS	Present
FM 18	20-04-2022	HVS	Present
FM 14	21-04-2022	Urine	Present
FM 3	13-05-2022	Urine	Present
FM 4	18-05-2022	Urine	Present
FM 5	18-05-2022	HVS	Present
FM 16	20-05-2022	Urine	Present
FM 13	08-03-2022	Urine	Present
FM 17	08-03-2022	HVS	Present

Key: HVS- High Vaginal Swab

Escherichia coli is a bacterium commonly found in the human body. It exists as a commensal in the body and the harmless strains are found in the gut microflora and prevent colonization by pathogenic organisms. Pathogenic *E. coli* enters the body principally through the fecal oral route, and could cause a number of life-threatening diseases such as diarrhea, hemorrhagic colitis (bloody diarrhea), and rare cases of hemolytic uremic syndrome (HUS) which affects the kidneys and blood clotting function in humans. Very recently, multidrug-resistant *E. coli* was reported to be found from clinical isolates from a hospital in Khartoum State, Sudan by Ibrahim et al., (2012), necessitating the need for more investigation across healthcare facilities worldwide. The current study was therefore aimed at investigating the

prevalence of multidrug resistant *E. coli* in isolates obtained from out-patients that attend Faith Mediplex hospital, Benin City.

In the current study, *E. coli* O157:H7 was reported as the principal organism from patients in Faith Mediplex, Benin City, Edo State, Nigeria. This bacterium is highly pathogenic, and is principally found in the gut and feces of cattle (Ameer et al., 2022). It can be transmitted through the fecal–oral route after ingestion of contaminated, undercooked food, water and from person to person through fecal shedding (Ameer et al., 2022). According to Ameer et al. (2022), this organism contributes to the development of hemolytic uremic syndrome which is a secondary illness to the production of the Shiga–toxin which causes gastrointestinal illnesses and bloody diarrhea. According to Lim et al. (2010), the infectious dose for this strain of *E. coli* is less than 10 cfu/mL, making it of potential public health concern. The results of the current study, is in keeping with that of Ngbede et al. (2007), who reported relatively high prevalence of this pathogen in patients with diarrheal stool in University of Jos Teaching Hospital, Plateau State, Nigeria, and was higher in children between the ages of 6-10, and adults between 51-60 years. Although the age of out-patients was not put into consideration in this study, it would be difficult to postulate if the prevalence of this bacterium is also dependent on age group, in Benin City. Ngbede et al. (2007) further attributed the isolation of *Escherichia coli* O157:H7 in patients to be due to the consumption of undercooked food especially from bovine (cattle) sources. In another study conducted in Morogoro Municipality, Eastern Tanzania, vero-cytotoxigenic *E. coli* O157:H7 were isolated from fecal samples gotten randomly from patients in Morogoro Regional Hospital. This infection was linked to poor sanitation, poor personal hygiene, improper sewage and refuse disposal (Raji et al., 2008). Omoruyi and Ojubiya (2022) recently reported pathogenic strains of *E. coli* from market dumpsites in Edo South Senatorial district, South-South Nigeria. Elsewhere, *E. coli* O157:H7 was isolated in children with diarrhea in Eastern Ethiopia (Getaneh et al., 2020), suggesting that this bacterium is ubiquitous in the Hospital environment, although only limited studies are reported in literature, probably because *E. coli* are underestimated to be present in Hospital environment, and sick patients. This also explains why several studies on ready-to-eat foods, vegetables and beef are available in literature (Enabulele and Uraih, 2009; Reuben and Makut, 2014; Zhang et al., 2015; Keraye et al., 2019). Keraye et al. (2019) reported *E. coli* O157:H7 in freshly harvested vegetable spinach from different markets in Zaria, Kaduna State. The contamination was due to the water used to irrigate the vegetable farms. The water was from a river contaminated with human and animal feces. Enabulele and Uraih (2009), reported the presence of this pathogen in meat samples from abattoir. They further reported that the pathogen found was due to the poor sanitary environment where the animals were killed and sold.

Table 2. Cultural, Morphological and Biochemical Characteristics of *Escherichia coli* Isolated from Patients

Isolates	Color	Gram-stain	Shape	Catalase	Citrate	H ₂ S	Indole	Mannitol	Lactose	Fructose	Glucose
FM 1	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 2	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 3	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 4	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 5	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 6	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 7	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 8	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 9	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 10	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 11	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 12	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 13	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 14	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 15	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 16	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 17	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
FM 18	Green metallic sheen	-ve	rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve

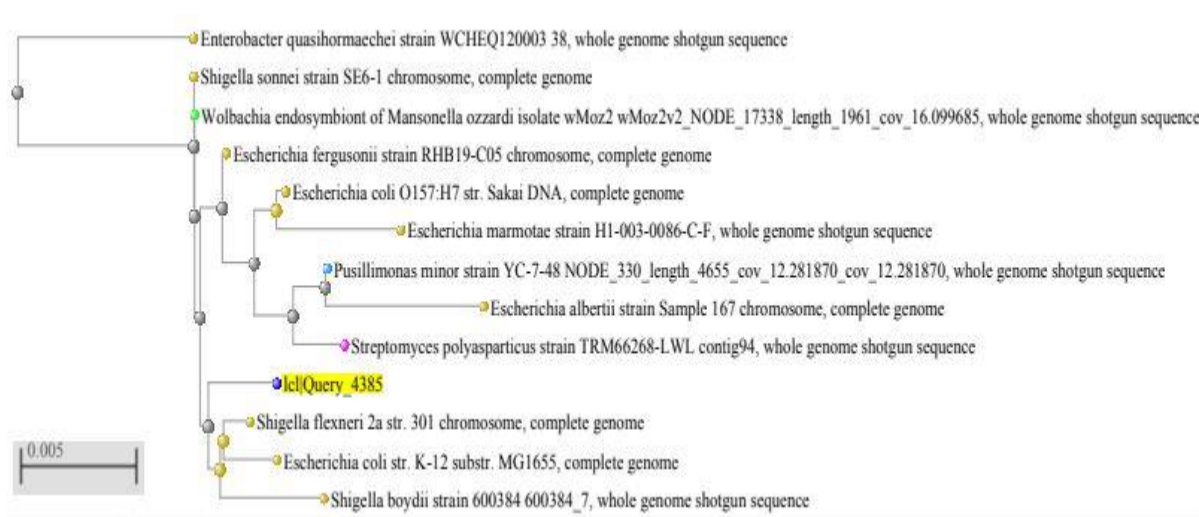


Fig. 1 BLAST representation of 16S rRNA analysis of bacterial isolate

Table 3. 16S rRNA Analysis of the Bacterial Isolate

Confirmed Isolate	Query cover	E – value	%identity
<i>Escherichia coli</i> O157:H7 strain Sakai	97%	0.0	99.08%

Table 4. Antibiotics Susceptibility Profile of *E. coli* Isolated from Patients at Faith Mediplex Hospital

Isolates	CXM	CRO	CTX	ZEM	OFX	GN	NA	LBC	AUG	ACX	IMP	NF	S (%)	I (%)	R (%)
FM 1	S	S	S	S	S	S	R	S	I	S	S	S	83.3%	8.3%	8.3%
FM 2	S	S	S	S	S	S	S	S	S	S	S	S	100%	0%	0%
FM 3	R	S	R	R	S	R	R	S	R	R	R	S	33.3%	0%	66.6%
FM 4	R	I	R	R	I	S	R	R	R	R	R	S	16.6%	16.6%	75.0%
FM 5	R	S	R	R	S	S	R	R	R	R	R	S	33.3%	0%	66.6%
FM 6	R	S	R	R	I	S	R	R	R	R	R	S	25.0%	8.3%	66.6%
FM 7	R	S	S	S	R	R	R	R	R	R	S	S	41.6%	0%	58.3%
FM 8	R	S	I	I	I	I	R	R	R	R	I	S	16.6%	41.6%	41.6%
FM 9	R	S	R	R	I	R	R	R	R	R	R	I	8.3%	16.6%	75.0%
FM 10	R	S	R	R	I	R	R	S	R	R	R	S	25.0%	8.3%	66.6%
FM 11	S	S	S	S	R	R	R	R	R	R	R	R	33.3%	0%	66.6%
FM 12	S	S	S	S	S	S	R	S	S	S	R	S	83.3%	0%	16.6%
FM 13	S	S	S	S	S	S	R	S	S	I	R	S	75.0%	8.3%	16.6%
FM 14	R	S	R	R	S	S	R	R	R	R	R	S	33.3%	0%	66.6%
FM 15	R	S	R	R	I	R	R	R	R	R	R	S	16.6%	8.3%	75.0%
FM 16	R	S	R	R	I	S	R	R	R	R	R	S	25.0%	8.3%	58.3%
FM 17	S	S	S	S	S	S	R	S	R	S	R	S	75.0%	0%	25.0%
FM 18	S	S	S	S	S	S	R	S	S	S	S	S	91.6%	0%	8.3%

Key: Amoxicillin clavulanate (AUG), Cefotaxime (CTX), Imipenem/Cilastatin (IMP), Ofloxacin (OFX), Gentamycin (GN), Nalidixic acid (NA), Nitrofurantoin (NF), Cefuroxime (CXM), Ceftriaxone Sulbactarm (CRO), Ampiclox (ACX), Cefexime (ZEM), Levofloxacin (LBC), Susceptible (S), Resistant (R), Intermediate (I).

Table 5. Multidrug Resistance Profile of *Escherichia coli* Isolated from Out-patients Attending Faith Mediplex hospital

Isolates	MDR Profile	MDR INDEX
FM 7	CXM, GN, NA, AUG, ACX, OFX, OX	0.50
FM 11	GN, OFX, NA, LBC, AUG, ACX, IMP, NF	0.57
FM 10	CXM, CTX, ZEM, GN, NA, AUG, ACX, IMP	0.57
FM 14	CXM, CTX, ZEM, NA, AUG, ACX, IMP, LBC	0.57
FM 1	CXM, CTX, ZEM, GN, NA, AUG, ACX, IMP, OX	0.64
FM 6	CXM, CTX, ZEM, GN, NA, AUG, ACX, IMP, OX	0.64
FM 8	CXM, CTX, ZEM, NA, LBC, AUG, ACX, IMP, OX	0.64
FM 15	CXM, CTX, ZEM, GN, NA, LBC, AUG, ACX, IMP	0.64
FM 16	CXM, CTX, ZEM, NA, LBC, AUG, ACX, IMP, OX	0.64
FM 4	CXM, CTX, ZEM, GN, NA, AUG, ACX, IMP, OX, LBC	0.71
FM 5	CXM, CTX, ZEM, GN, NA, AUG, ACX, IMP, OX, LBC	0.71
FM 9	CXM, CTX, ZEM, GN, NA, LBC, AUG, ACX, IMP, OX	0.71

Key: AUG: Amoxicillin clavulanate; CTX: cefotaxime; IMP: Imipenem/cilastatin; OFX: ofloxacin; GN: gentamycin; NA: nalidixic acid; NF: nitrofurantoin; CXM: cefuroxime; CRO: ceftriaxone culbactarm; ACX: ampiclox; ZEM: cefexime; LBC: Levofloxacin; OX: Oxacillin

Table 6. Phenotypic detection of methicillin and colistin-resistant *E. coli* from patients' samples

Isolates	Oxacillin	Colistin
FM 1	R	S
FM 2	R	S
FM 3	R	S
FM 4	R	S
FM 5	R	S
FM 6	R	S
FM 7	R	S
FM 8	R	S
FM 9	R	S
FM 10	S	S
FM 11	S	S
FM 12	S	S
FM 13	S	S
FM 14	S	S
FM 15	S	S
FM 16	R	S
FM 17	S	S
FM 18	R	S

Key: R: Resistant; S: Susceptible

Table 7. Detection of Virulence Gene in *Escherichia coli* Isolated from Patients' samples from Faith Mediplex hospital

Isolates	<i>uidA</i>	<i>Lt</i>	<i>Stx</i>	<i>fliCH7</i>
FM 1	-	-	-	-
FM 2	-	-	-	-
FM 3	-	-	-	-
FM 4	-	-	-	-
FM 5	+	-	-	-
FM 6	-	-	-	-
FM 7	-	-	-	-
FM 8	-	+	-	-
FM 9	-	-	-	-
FM 10	-	-	-	-
FM 11	-	-	-	-
FM 12	+	-	-	-
FM 13	-	-	-	-
FM 14	-	-	-	-
FM 15	-	-	-	-
FM 16	+	+	-	-
FM 17	-	-	-	-
FM 18	+	-	-	-

Key: Positive: +; Negative: -

This pathogen has different virulence factors that have been shown to be very crucial to its ability to cause diseases in humans. The most important factor it possesses is its ability to produce the shiga – toxin. There are two forms of this toxin *stx1* and *stx2* which are coded for by the *stx1* and *stx2* genes. These forms have been reported to be associated with the

development of hemolytic uremic syndrome (HUS) (Lupindu, 2017). *Stx1* is made up of three subtypes, *stx1a*, *stx1c*, and *stx1d* and *stx2* is divided into seven subtypes *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g*. Severe HUS is caused by the subtypes of *stx2* (Lupindu, 2017). The shiga – toxin binds to the eukaryotic surface of the cells, causing the inhibition of protein synthesis and the death of the hosts' cell. Another virulence factor that has been reported to be expressed by this pathogen is the production of intimin by the *eae* gene. Intimin which is also implicated with the enteropathogenic *E. coli* helps the bacteria to attach itself to the intestinal epithelia during its colonization causing lesions to form and diarrhea (Lupindu, 2017). Another virulence factor associated with this organism is the production of the toxin enterohaemolysin. This toxin destroys the red blood (erythrocyte) cell membrane. Sometimes this toxin is used as a tool to detect the presence of this pathogen by confirmation of the *ehxA* gene (Lupindu, 2017).

In this study, the antibiotics used were amoxicillin clavulanate, cefotaxime, imipenem/cilastatin, ofloxacin, gentamycin, nalidixic acid, nitrofurantoin, cefuroxime, ceftriaxone sulbactam, ampiclox, cefexime, levofloxacin, oxacillin and colistin. The isolates were mostly resistant to nalidixic acid, amoxicillin clavulanate, ampiclox, oxacillin and levofloxacin, and the result is slightly similar to a study carried out by Kibret and Abera, (2011) which shows that the isolates from clinical sources in North-East Ethiopia were highly resistant to erythromycin, amoxicillin and tetracycline and sensitive to nitrofurantoin, norflaxocin, gentamicin and ciprofloxacin. Based on their study they suggested the four most sensitive antibiotics listed above be used as empirical treatment for *E. coli* infection. In this study, it shows that all isolates except one was susceptible to nitrofurantoin. Antibiotics showing high susceptibility patterns in this study were colistin, ceftriaxone sulbactam, nitrofurantoin, gentamycin and ofloxacin. Virulent genes such as heat-labile toxins [*Lt*] and Beta – glucuronidase [*uidA*] were found in the isolates in this study. Omoruyi and Ojubiaja (2022) also indicated the presence of these virulent genes in *E. coli* isolated from market dumpsites in Edo South Senatorial District, Nigeria. In this study, all the *E. coli* isolates were *stx* and *fliCH7* negative. The occurrence of this pathogen in clinical isolates could be due to contamination from food or water or improper sanitation and hygiene which is a serious matter of public health concern.

Conclusions

This study shows that multidrug-resistant *E. coli* and pathogenic *E. coli* O157:H7 is found in clinical isolates from outpatients attending Faith Mediplex Hospital, Benin City, Edo State. Hence, there is a need to incorporate antibiotic stewardship programs for healthcare facilities to reduce multidrug resistance, educate individuals on proper hygiene and sanitation and regular testing for the presence of these organisms because of the life-threatening diseases they can cause.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Ethical Considerations

The study was approved by the institutional ethical committee.

Funding support

The author declares that they have no funding support for this study.

References

- Ahmed, M. A. E. E., Zhong, L., Shen, C., Yang, Y., Doi, Y. and Tian, G. (2020). Colistin and its role in the era of antibiotic resistance: an extended review (2000-2019). *Emerging Microbes Infection*, 9(1), 868-885.
- Ameer, M. A., Wasey, A. and Salen, P. (2022). *Escherichia coli* (E. coli O157 H7): In: StatPearls, Treasure Island (FL): StatPearls Publishing. 631.
- Borowiak, M., Baumann, B., Fischer, J., Thomas, K., Deneke, C., Hammerl, J. A., Szabo, I. and Malorny, B. (2020). Development of a novel *mcr-6* to *mcr-9* multiplex PCR and assessment of *mcr-1* to *mcr-9* occurrence in colistin-resistant *Salmonella enterica* isolates from environment, feed, animals and food (2011-2018) in Germany. *Frontiers in Microbiology*. 11, 80-88.
- Borowiak, M., Hammerl, J. A., Deneke, C., Fischer, J., Szabo, I., and Malorny, B. (2019). Characterization of *mcr-5*-harboring *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from animal and food origin in Germany. *Antimicrobial Agents and Chemotherapy*, 63:e00063-19. doi: 10.1128/AAC.00063-19.
- Cannatelli, A., D'Andrea, M. M., Giani, T., Pilato, V. D., Arena, F., Ambretti, S., Gaibani, P. and Rossolini, G. M. (2013). In vivo emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. *Antimicrobial Agents and Chemotherapy*. 57(11), 5521-5526.
- Carroll, L. M., Gaballa, A., Guldemann, C., Sullivan, G., Henderson, L. O. and Wiedmann, M. (2019). Identification of novel mobilized colistin resistance gene *mcr-9* in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. *MBIO*. 7;10(3):e00853-19.
- Chavda, K. D., Westblade, L. F., Satlin, M. J., Hemmert, A. C., Castanheira, M., Jenkins, S. G., Chen, L. and Kreiswirth, B. N. (2019). First report of *bla_{VIM-4}*- and *mcr-9* coharboring *Enterobacter* species isolated from a pediatric patient. *Clinical Science and Epidemiology*, 4(5), e00629-19.
- Clinical and Laboratory Standards Institute [CLSI] (2020). *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. CLSI supplement M100. Wayne, PA, 332 pp.
- Dadashi, M., Sameni, F., Bostanshirin, N., Yaslianifard, S., Khosravi-Dehaghi, N., Nasiri, M. J., Goudarzi, M., Hashemi, A. and Hajikhani, B. (2021). Global prevalence and molecular epidemiology of *mcr*-mediated colistin resistance in *Escherichia coli* clinical isolates: a systematic review. *Journal of Global Antimicrobial Resistance*. 29, 444-461.
- Enabulele, S. A. and Uraih, N. (2009). Enterohaemorrhagic *Escherichia coli* O157:H7 prevalence in meat and vegetables sold in Benin City, Nigeria. *African Journal of Microbiology Research*, 3(5), 276-279.
- Falagas, M. E., Rafailidis, P. I., Ioannidou, E., Alexiou, V. G., Matthaiou, D. K., Karageorgopoulos, D. E., Kapaskelis, A., Nikita, D. and Michalopoulos, A. (2009). Colistin therapy for microbiologically documented multidrug-resistant Gram-negative bacterial infections: a retrospective cohort study of 258 patients. *International Journal of Antimicrobial Agents*. 35(2), 194-199.
- Gelbicova, T., Barakova, A., Florianova, M., Jamborova, I., Zelendova, M., Pospisilova, L., Kolackova, I. and Karpiskova, R. (2019). Dissemination and comparison of genetic determinants of *mcr*-mediated colistin resistance in enterobacteriaceae via retail raw meat products. *Frontiers in Microbiology*. 10, 2824.
- Getaneh, D. K., Hordofa, L. O., Ayana, D. A., Tessema, T. S. and Regassa, L. D. (2021). Prevalence of *Escherichia coli* O157:H7 and associated factors in under-five children in Eastern Ethiopia. *PLoS One*, 16(1), e0246024.
- Giske, (2015). Contemporary resistance trends and mechanisms for the old antibiotics colistin, temocillin, fosfomycin, mecillinam and nitrofurantoin. *Clinical Microbiology and Infection*, 21(10), 899-905.
- Ibrahim, M. E., Bilal, N. E. and Hamid, M. E. (2012). Increased multi-drug resistant “*Escherichia coli*” from hospitals in Khartoum state, Sudan. *African Health Sciences*, 12(3), 368-375.
- Karaye, G. P., Karaye, K. K. and Kaze, P. D. (2019). Detection of *Escherichia coli* in freshly harvested spinach samples collected from five different markets in Zaria. *American Journal of Biomedical Science and Research*, 4(2),1-7.
- Kibret, M. and Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Science*, 11(1), S40-45.
- Lim, J. Y., Yoon, J. W. and Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*. 20(1), 5-14.
- Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X. and Yu, L. F. (2016). Emergence of Plasmid-Mediated Colistin Resistance Mechanism *mcr-1* in Animals and Human Beings in China: A Microbiological and Molecular Biological Study. *The Lancet Infectious Diseases*. 16(2), 161 – 168.
- Luo, Q., Yu, Wei, Zhou, K., Guo, L., Shen, P., Lu, H., Huang, C., Xu, H., Xu, S., Xiao, Y. and Li, L. (2017). Molecular epidemiology and colistin resistant mechanism of *mcr*- positive and *mcr*-negative clinical isolated *Escherichia coli*. *Frontiers in Microbiology*. 17(8), 2262.
- Lupindu, A. M. (2018). Epidemiology of shiga toxin-producing *Escherichia coli* O157:H7 in Africa in review. *Southern African Journal of Infectious Diseases*. 33(1), 24-30.
- Madni, W. A., Mohsin, M., Nawaz, Z., Muzammil, S., Zahoor, M. A. and Asif, R. (2022). Molecular mechanism of antimicrobial co-resistance colistin (*mcr-1*) and ESBLs genes among *Escherichia coli* isolates from commercial chickens in Pakistan. *Brazilian Journal of Biology*. 84. E267494.
- Marchaim, D., Chopra, T., Pogue, J. M., Perez, F., Hujer, A. M., Rudin, S., Endimiani, A., Navon-Venezia, S., Hothi, J., Slim, J., Blunden, C., Shango, M., Lephart, P. R., Salimnia, H., Reid, D., Moshos, J., Hafeez, W., Bheemreddy, S., Chen, T. Y., Dhar, S., Bonomo, R. A. and Kaye, K. S. (2011). Outbreak of colistin-resistant, carbapenem-resistant *Klebsiella pneumoniae* in

- metropolitan Detroit, Michigan. *Antimicrobial Agents and Chemotherapy*. 55(2), 593-599.
- Munita, J. M. and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology Spectrum*, 4(2), 1-37.
- Ngede, J. E., Jideani, A. I. O. and Agbo, E. B. (2007). Comparison of enterovirulent *E. coli* (EVEC) with other bacterial enteropathogens isolated from diarrhoeal specimens in Jos hospitals, Nigeria. *Journal of Food Agriculture and Environment*, 5(1), 30-33.
- Omoruyi, I. M. and Ojubiaja, S. E. (2022). Antibigram and virulence gene detection in *Escherichia coli* and *Vibrio* species isolated from market dumpsites in Edo South Senatorial District, Nigeria. *Science World Journal*. 17(1), 17-25.
- Omoruyi, I. M., Ovia, P. P., Ahmad-Dirisu, Z. and Osemwowa, E. (2023). Plasmid-borne mobilizable colistin resistant gene (*mcr-1*) detection and multidrug resistant bacteria isolated from abattoir environment. *Nigerian Journal of Biotechnology*.
- Raji, M. A., Minga, U. M. and Machan'U, R. S. (2008). Prevalence and characterization of verotoxin producing *Escherichia coli* O157 from diarrhea patients in Morogoro, Tanzania. *Tanzania Journal of Health Research*, 10(3), 151- 158.
- Reuben, R. C. and Makut, M. D. (2014). Occurrence of *Escherichia coli* O157:H7 in vegetables grown and sold in Lafia metropolis, Nigeria. *World Journal of Microbiology*, 1(3), 17-21.
- Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z. and Wang, Y. (2018). Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerging Microbes and Infection*. 7(1), 122.
- Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., Zhang, R., Walsh, T. R., Shen, J. and Wang, Y. (2017). Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBIO*. 15;8(4) e01166-17.
- Zhang, X., Zhang, B., Guo, Y., Wang, J., Zhao, P., Liu, J. (2019). Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu Province. *International Journal of Food Microbiology*. 291, 87–90.

How to cite this article

Lisk-Anani, E. A., Duru, M., Inegbedion, L. O. and Omoruyi, I. M. (2023). Prevalence of virulent, pathogenic and colistin resistant genes in *Escherichia coli* isolated from out-patients attending Faith Mediplex, Benin City, Edo state, Nigeria. *Microbial Science Archives*, Vol. 3(1), 43-51. <https://doi.org/10.47587/MSA.2023.3108>

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



Publisher's Note: MD International Publishing stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.