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Research Article

Mobilized Colistin Resistance (mcr) Genes, Resistotyping and Virulence Markers in *Escherichia coli* from Clinical and Water Samples

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ABSTRACT

Pathogenic *Escherichia coli* are responsible for a diverse range of human infections and diseases, causing a significant increase in morbidity and mortality rates. This study determined the antibiotic resistance, virulence markers, and mobilized Colistin resistance genes in *E. coli* using the disc diffusion method and the Vitek 2 automated system, bacteriological media, and multiplex polymerase chain reaction, respectively. The results showed that 40.0%, 23.1%, and 30.0% of urine, blood, and wound samples had *E. coli*, while 29.4% of water samples contained *E. coli*. The *E. coli* were highly sensitive (> 68.4%) to Ampicillin and Ofloxacin, but > 47.4% were resistant to Augmentin, Pefloxacin, and Nalidixic Acid. Of the 19 *E. coli* isolates, 9 (47.4%) and 8 (42.1%) exhibited intermediate resistance to Streptomycin, and Ciprofloxacin, respectively. All *E. coli* were resistant to Cefalexin, and 84.2% were resistant to Nalidixic Acid, while *E. coli* (Ec-B3) was sensitive to Ciprofloxacin. The results showed that *E. coli* (Ec-W1 and Ec-U5) and ExPEC (Ec-B1 and Ec-B2) shared 97.5% and 92% similarity, respectively. Of the 19 *E. coli* strains obtained, 69.4%, 57.9%, and 52.6% were gelatinase-, lipase-, and caseinase-producing strains, respectively. The results revealed that 57.9% of the *E. coli* strains were mobilized Colistin-resistant (COR), with 31.6%, 5.3%, and 21.1% having the mcr-1, mcr-2, and mcr-3 genes, respectively. The *E. coli* harboured some virulence markers and were highly resistant to Cefalexin, Nalidixic Acid, Augmentin, Pefloxacin, and Colistin, with mcr-1 as the predominant mcr gene. Thus, regular monitoring of antibiotic susceptibility of bacterial isolates in both the community and hospital settings is recommended.

Keywords: *Colistin, Antibiotic, Resistance, Escherichia coli, Pathogenic, Genes.*

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INTRODUCTION

Pathogenic *Escherichia coli*, the most common and versatile Gram-negative bacterial pathogens, are responsible for a diverse range of human infections and diseases, both in health care and community settings (Russo and Johnson, 2003; Akinjogunla *et al*., 2009; Odeyemi *et al*., 2019). Globally, *E. coli* infections and *E. coli*-induced diseases are causing a significant increase in morbidity and mortality rates, medical costs, and productivity losses (Smith *et al*., 2007). Pathogenic *E. coli* strains can cause pelvic inflammatory disease, bacteremia, neonate meningitis (Russo and Johnson, 2003; Kim, 2012), skin and soft tissue infections, hospital-acquired pneumonia (Russo and Johnson, 2003), infective peritonitis, urinary tract infections (UTIs), and other infections outside the gastrointestinal system or at non-intestinal sites.

Colistin has gained clinical value as a last-resort antibiotic for the treatment of severe bacterial infections, especially infections caused by multidrug-resistant pathogenic *E. coli* (Zafer *et al*., 2019). However, the current emergence of

Colistin resistance among pathogenic *E. coli* strains poses a serious clinical challenge and represents a major impact on public health. The Colistin resistance is predominantly attributed to the presence of the Colistin-resistance (mcr) gene (Sonnevend *et al*., 2016) and the modification of lipid A, which attaches the lipopolysaccharide molecule to the outer membrane, leading to a reduction in the affinity for the antibiotic (Anjum *et al*., 2016).

Virulence and antibiotic resistance in pathogenic *E. coli* are inextricably related to the phylogenetic background of the strain and play a significant role in the infection process. The production of toxin, iron acquisition factors, extracellular hydrolytic enzymes (EHEs), and antigenic modulations are important virulence-associated factors that enhance the pathogenicity of pathogenic *E. coli* (Köhler and Dobrindt, 2011). The EHEs, such as proteases, phospholipases, haemolysins, and lipases, aid in adherence, tissue penetration, invasion, and destruction of host tissues (Akinjogunla *et al*., 2019). In this study, the prevalence, antibiotic susceptibility profile, virulence factors, and Colistin-resistance (mcr) gene among pathogenic *E. coli* were determined.

MATERIALS AND METHODS

Collection of Samples: A total of 75 samples, comprising mid-stream urine (n = 25), blood (n = 17), wound swabs (n = 14), and water (n = 19), were collected using sterile leak-proof, screw-cap containers and swab sticks in Uyo, Akwa Ibom State, between November and December, 2021. The clinical samples were collected from patients attending different private and government hospitals. All samples were transported in an icebox to the Microbiology Laboratory for bacteriological analyses.

Bacteriological Analysis of Samples: One millilitre (1 mL) of each well-mixed mid-stream urine sample was inoculated onto plates of Cysteine Lactose Electrolyte Deficient Agar (CLED). Each wound swab was separately dipped into a test tube containing sterile distilled water (9 mL), and 0.1 mL was pipetted and surface-inoculated on to plates of Eosine Methylene Blue (EMB) Agar and Blood Agar (BA). Each blood sample (1 mL) was inoculated into 5 mL of Brain Heart Infusion Broth and incubated. After incubation, 1 mL of the inoculated broth medium was subcultured onto a plate of BA. One millilitre (1 mL) of each well-mixed water sample was inoculated onto plates of EMB agar. All the plates were incubated at 37°C for 24 h. After incubation, a loopful of a single colony was streaked onto each freshly prepared nutrient agar plate and incubated at 37°C for 24 h. A pure colony of the isolate was streaked onto the slant surface of nutrient agar contained in sterile McCartney bottles and incubated at 37°C for 24 h, stored at 4°C prior. All pure isolates were Gram stained, and identified using the VITEK 2 automated system (Biomeriux Inc., France).

Antibiotic Susceptibility Profile of *E. coli*: The antibiotic susceptibility profiles of *E. coli* were determined by Kirby Bauer disc diffusion technique (Akinjogunla *et al*., 2011; CLSI, 2018). Briefly, 10 µL of each *E. coli*, adjusted to the 0.5 McFarland Standard, was streaked homogenously onto each Petri dish containing MHA using a sterile cotton swab and allowed to dry for 5 min. The discs impregnated with antibiotics: Ciprofloxacin (5 µg), Cotrimoxazole (25 µg), Gentamicin (30 µg), Cefalexin (30 µg), Ampicillin (10 µg), Ofloxacin (5 µg), Augmentin (30 µg), Streptomycin (10 µg), Pefloxacin (5 µg) and Nalidixic Acid (5 µg) (Oxoid, UK) were aseptically placed on the plates. The plates were inverted and incubated at 37oC for 16 h. The inhibition zones around the antibiotic discs were measured to the nearest millimeter. The interpretation of the measurement as either sensitive, intermediate, or resistant was made using the interpretative manual by CLSI (2018).

Detection of Colistin Resistance among *E. coli*: Briefly, 10 µL of each *E. coli*, adjusted to the 0.5 McFarland Standard, was streaked homogenously onto each plate of MHA. A disc impregnated with Colistin (10 µg, COL) was placed on a MHA plate inoculated with 10 µL of *E. coli*. The plates were inverted and incubated at 37oC for 16 h. The inhibition zones around the antibiotic discs were measured to the nearest millimeter after incubation, and a growth-inhibitory zone of ≤ 10 mm was considered Colistin resistance and selected for further testing for Colistin-resistant genes.

Extraction of genomic DNA of *E. coli*: The genomic DNA (gDNA) of 11 colistin-resistant *E. coli* was extracted by the boiling method. Briefly, 100 µL of an overnight freshly grown culture was centrifuged at 2,300 x g for 5 min. The supernatant was discarded, and the pellet was re-suspended in 100 µL of phosphate buffer solution, boiled in a water bath at 100 oC for 10 min, cooled on ice for 10 min in each step, and centrifuged at 2,300 x g for 10 min. The supernatant containing gDNA was collected, stored at –20 oC in an Eppendorf tube, and later used as a DNA template.

PCR Amplification of Colistin-Resistant Genes in *E. coli*: The multiplex polymerase chain reaction (PCR) amplification assay was used for detection of the Colistin-resistant genes (mcr-1, mcr-2, and mcr-3) in isolates was carried out with slight modification using the specific primers presented in Table 1 (Amna *et al*., 2021). A final PCR reaction volume of 12.5 µL contained 6.25 µL of PCR-master mix, 0.25 µL of each primer (0.2 pmol/ µL), 1 µL of template DNA, and 4.75 µL of nuclease-free water. The automated thermal cycler was used for PCR amplification. The PCR amplification conditions were as follows: initial denaturation at 94°C for 10 min., followed by 35 cycles of denaturation at 94°C for 30 sec., annealing temperature at 60°C for 1 min., extension at 72°C for 1 min., and a final extension at 72°C for 10 min. The amplified PCR products were electrophoresed on a 1.5% agarose gel containing 0.5 µg/mL of ethidium bromide. Thereafter, the amplified PCR products were visualized under a UV transilluminator.

Table 1:
Primers used to assess the Colistin-Resistant Genes

Target Genes	Primer	Sequence (5′→3′)	Amplicon Size (bp)	References
mcr-1	F	GTGTGGTACCGACGCTCGG	460	Eiamphungporn *et al*. (2018)
	R	CAAGCCCAATCGGCGCATC		
mcr-2	F	AAGTGTGTTGGTCGCAGTT	715	Rebelo *et al*. (2018)
	R	TCTAGCCCGACAAGCATACC		
mcr-3	F	AAATAAAAATTGTTCCGCTTATG	929	Borowiak *et al*. (2017)
	R	AATGGAGATCCCCGTTTTT		

Determination of Lipase, Caseinase and Gelatinase Producing *E. coli*: The lipase, caseinase and gelatinase producing *E. coli* were detected using tributyrin, skimmed milk and gelatin agar, respectively. The plates of tributyrin, skimmed milk and gelatin agar were spot inoculated with *E. coli* and incubated at 35ºC for 24 h. Clear zones around the colonies indicated production of
lipase, caseinase and gelatinase (Akinjogunla *et al*., 2017).

Detection of Amylase, Lecithinase and Haemolysin Producing *E. coli*: Amylase, lecithinase and haemolysin producing *E. coli* was detected using starch agar, egg yolk agar and blood agar, respectively. The *E. coli* were streaked onto plates of starch, egg yolk and blood agar, and incubated at 35ºC for 24 h. After incubation, 3 drops of 10% Lugol's Iodine were put on the culture plates of starch agar and allowed to react for 10 min. Amylase, lecithinase and haemolysin production was indicated by clear zones around the colonies (Akinjogunla *et al*., 2017).

Statistical Analysis: The Statistical Package for Social Sciences (IBM SPSS, Window Software Version 22.0, Armonk, NY: IBM Corp.) was used for analysis of the data.

RESULTS

The morphological and biochemical characteristics, enzymatic reaction (ONPG, ornithine decarboxylase, alkaline phosphatase, acetate utilization), and sugar fermentation test of ExPEC from blood, wound, urine and water samples are presented in Table 2. A total of nineteen (19) *E. coli* were obtained from blood, wound, urine and water samples. Of the 19 water samples collected, 5 (26.3%) had *E. coli*. The results showed that 17.6% and 21.4% of blood and wound samples had *E. coli*, while 32.0% (8) of urine samples contained *E. coli* (Table 3).

Table 2:
Morphological and Biochemical Characteristics, Enzymatic Reaction of *E. coli*

Gram reaction		Coagulase	Catalase	Oxidase	Vogues Proskauer	Methyl Red	Indole	Urease	Motility	Citrate	Acetate Utilization	Alkaline Phosphatase	ONPG	Ornithine Dec.	Fructose	Raffinose	Mannitol	Maltose	Galactose	Lactose	Glucose	Sucrose	Probable Bacteria / Code	
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+	-	+	+	+	+	*Escherichia coli*	Ec-W1
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+	+	+	*Escherichia coli*	Ec-W2
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	-	*Escherichia coli*	Ec-W3
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	-	*Escherichia coli*	Ec-W4
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+	-	+	+	+	+	*Escherichia coli*	Ec-W5
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+	+	-	*Escherichia coli*	Ec-U1
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-U2
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-U3
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-U4
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+	+	+	*Escherichia coli*	Ec-U5
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-U6
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	-	*Escherichia coli*	Ec-U7
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	-	*Escherichia coli*	Ec-U8
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+	-	+	+	+	-	*Escherichia coli*	Ec-Ws1
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-Ws2
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-Ws3
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	+	*Escherichia coli*	Ec-B1
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	-	*Escherichia coli*	Ec-B2
-	rod	-	+	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	+	+	+	*Escherichia coli*	Ec-B3

Key: Ec-W1-Ec-W5: *E.coli* from Water Samples; Ec-U1-Ec-U8: *E.coli* from Urine Samples; Ec-Ws1-Ec-Ws3: *E.coli* from Wound Samples; Ec-B1-Ec-B3: *E.coli* from Blood Samples. ONPG: Ortho-Nitrophenyl-β-Galactoside; Ornithine Dec.: Ornithine Decarboxylase

Table 3:
Specimen wise Distribution of *E. coli*

Samples	No of Samples Collected	No. of *E. coli* Isolated	% of *E. coli* Isolated
Urine	25	8	32.0
Blood	17	3	17.6
Wound	14	3	21.4
Water	19	5	26.3
Total	75	19	25.3

The results showed marked variations in antibiotic sensitivities among the *E. coli* from the urine, wound, water, and blood samples (Table 4). The *E. coli* were highly sensitive to Ampicillin, Cotrimoxazole, Gentamycin, and ofloxacin, with percentages of sensitivity ranging from 68.4% to 89.5%. The *E. coli* (*E. coli* Ec-W3, Ec-W4, and Ec-U4) were sensitive to Gentamycin, Ofloxacin, Ampicillin, and Ciprofloxacin; > 47.4% of the *E. coli* were resistant to Augmentin, Pefloxacin, and Nalidixic Acid. Of the 19 *E. coli* isolated, 7 (36.8%), 5 (26.3%), 9 (47.4%), and 8 (42.1%) exhibited intermediate resistance to Augmentin, Ampicillin, Streptomycin, and Ciprofloxacin, respectively.

Table 4:
Antibiotic Susceptibility Profile of *E. coli*

Code	Antibioitc Susceptibility / Zone of Inhibition (mm)									
	AU	CN	PEF	NA	OFX	CEP	PN	S	SXT	CPX
Ec-W1	R_{13}	S_{21}	R_{7}	R_{0}	S_{20}	R_{10}	S_{15}	S_{15}	S_{17}	I_{20}
Ec-W2	R_{13}	S_{21}	R_{7}	R_{0}	S_{19}	R_{10}	S_{14}	S_{15}	S_{16}	I_{20}
Ec-W3	I_{16}	S_{20}	R_{10}	I_{17}	S_{19}	R_{9}	S_{14}	S_{15}	S_{16}	S_{23}
Ec-W4	I_{15}	S_{20}	R_{10}	I_{16}	S_{17}	R_{9}	S_{15}	I_{14}	I_{14}	S_{21}
Ec-W5	I_{17}	S_{21}	R_{7}	I_{15}	S_{20}	R_{11}	S_{14}	S_{15}	S_{16}	I_{20}
Ec-B1	R_{12}	S_{16}	R_{15}	R_{10}	S_{16}	R_{8}	I_{12}	S_{15}	S_{17}	I_{18}
Ec-B2	R_{12}	I_{14}	I_{18}	R_{13}	R_{9}	R_{10}	R_{11}	S_{19}	S_{19}	S_{23}
Ec-B3	R_{10}	I_{14}	I_{20}	R_{10}	R_{12}	R_{8}	R_{10}	I_{12}	R_{11}	S_{23}
Ec-Ws1	R_{10}	S_{17}	R_{15}	R_{10}	S_{17}	R_{0}	I_{13}	I_{14}	S_{16}	I_{20}
Ec-Ws2	S_{20}	S_{19}	I_{20}	R_{12}	S_{20}	R_{0}	I_{13}	I_{14}	R_{10}	S_{23}
Ec-Ws3	S_{18}	S_{18}	S_{23}	R_{10}	S_{23}	R_{0}	I_{13}	S_{15}	R_{11}	S_{21}
Ec-U1	I_{15}	S_{18}	R_{14}	R_{0}	S_{20}	R_{8}	S_{15}	I_{12}	S_{20}	S_{22}
Ec-U2	I_{16}	S_{24}	R_{14}	R_{0}	S_{19}	R_{8}	I_{13}	R_{10}	S_{19}	S_{24}
Ec-U3	I_{15}	S_{15}	I_{18}	R_{0}	I_{15}	R_{0}	S_{15}	I_{12}	S_{18}	S_{23}
Ec-U4	S_{19}	S_{22}	R_{14}	R_{0}	S_{23}	R_{10}	S_{15}	I_{12}	S_{18}	S_{23}
Ec-U5	R_{13}	S_{21}	R_{0}	R_{0}	S_{20}	R_{10}	S_{14}	S_{15}	S_{16}	I_{20}
Ec-U6	R_{11}	S_{17}	S_{22}	R_{10}	S_{21}	R_{0}	S_{15}	R_{10}	S_{17}	S_{23}
Ec-U7	R_{12}	S_{23}	S_{24}	R_{13}	I_{14}	R_{10}	S_{16}	I_{12}	S_{16}	I_{20}
Ec-U8	I_{17}	S_{20}	R_{7}	R_{13}	S_{20}	R_{11}	S_{14}	I_{12}	S_{16}	I_{18}
Rate (%)	47.4	0.0	63.2	84.2	10.5	100	10.5	10.5	15.8	0.0

Keys: AU: Augmentin; CN: Gentamycin; PEF: Pefloxacin; NA: Nalidixic Acid; OFX: Ofloxacin; CEP: Cefalexin; PN: Ampicillin; SXT: Cotrimoxazole; S: Streptomycin; CPX: Ciprofloxacin; NZ: No zone of Inhibition; R: Resistant; I: Intermediate; S: Sensitive.

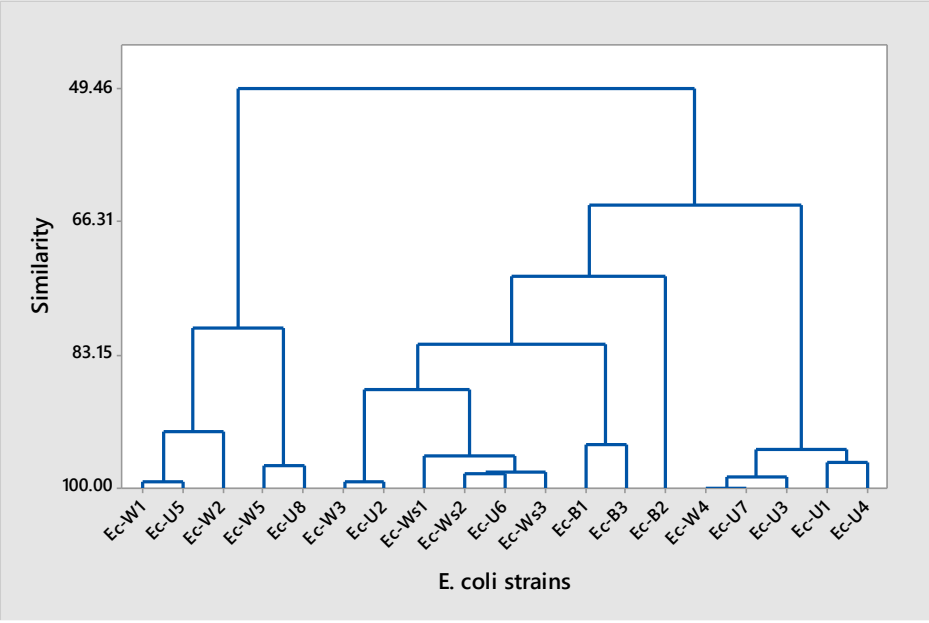

Figure 1:
The Relationship among *E. coli* based on Antibiotic Resistance and Inhibition Zones

All *E. coli* were resistant to Cefalexin, and 84.2% were resistant to Nalidixic Acid, while *E. coli* (Ec-B3) was only sensitive to Ciprofloxacin (Table 4).

Of the 19 *E. coli* isolated from urine, wound, water, and blood samples, 10.5% (2/19) *E. coli* displayed a resistant pattern of AU/NA/CEP/S, and 15.8% (3/19) *E. coli* displayed a resistant pattern of PEF/CEP. The most common pattern of antibiotic resistance among *E. coli* was AU/PEF/NA/CEP. *E. coli* (Ec-B2 and Ec-B3) displayed a resistance pattern of AU/NA/OFX/ CEP /PN/S, whereas *E. coli* (Ec-Ws2 and Ec-Ws3) were resistant to NA/CEP/SXT. The antibiotic resistance pattern of *E. coli* (Ec-U1, Ec-U4, and Ec-U8) was PEF/NA/CEP/S, while 2 (10.5%) *E. coli* (Ec-U2 and Ec-U3) showed NA/CEP/S as their resistant patterns (Table 5).

The results showed that *E. coli* (Ec-W1 and *E. coli* Ec-U5) shared 97.5% similarity; *E. coli* Ec-W3 and *E. coli* Ec-U2 also showed 97.5% similarity; there was a 92% similarity between *E. coli* Ec-B1 and *E. coli* Ec-B2, while *E. coli* (Ec-W3, Ec-U2, Ec-Ws2, Ec-Ws2, Ec-U6, and Ec-Ws3) shared 87.2% similarity (Fig 1). Of the 19 *E. coli* obtained, 69.4% (n = 13), 57.9% (n = 11), and 52.6% (n = 10) were gelatinase, lipase, and caseinase-producing strains. The results showed that between 26.3% and 42.1% of the *E. coli* were lecithinase, haemolysin, and amylase producers. *E. coli* (Ec-Ws2) possessed gelatinase, haemolysin, caseinase, lipase, and amylase; whereas *E. coli* (Ec-W3, *E. coli* Ec-B2, and *E. coli* Ec-U8) possessed only haemolysin, caseinase, and caseinase, respectively (Table 6). The results showed that 57.9% (n = 11) of the *E. coli* were Colistin-resistant (COR) strains, while 31.6%, 5.3%, and 21.1% of the *E. coli* tested had the colistin-resistant genes mcr-1, mcr-2, and mcr-3, respectively (Table 6).

Table 5:
Antibiotic Resistance Pattern of *E. coli*

Isolate Codes	Antibiotic Resistance Pattern	No of Occurence	% of Occurence
Ec-W1, Ec-W2, Ec-B1, Ec-Ws1, Ec-U5	AU/PEF/NA/CEP	5	26.3
Ec-U6, Ec-U7	AU/NA/CEP/S	2	10.5
Ec-W3, Ec-W4, Ec-W5	PEF/CEP	3	15.8
Ec-B2, Ec-B3	AU/NA/OFX/CEP/PN/S	2	10.5
Ec-Ws2, Ec-Ws3	NA/CEP/SXT	2	10.5
Ec-U1, Ec-U4, Ec-U8	PEF/NA/CEP/S	3	15.8
Ec-U2, Ec-U3	NA/CEP/S	2	10.5

Table 6:
Virulence Markers and Mobilized Colistin Resistance Gene in *E. coli*

	Virulence Markers						Colistin	Colistin Resistance Gene		
Codes	Gelatinase	Haemolysin	Caseinase	Lecithinase	Lipase	Amylase	Resistance	*mcr*-1	*mcr*-2	*mcr*-3
Ec-W1	+	+	-	-	+	+	+	+	-	-
Ec-W2	-	-	+	+	-	+	+	-	-	+
Ec-W3	-	+	-	-	-	-	-	-	-	-
Ec-W4	+	-	+	+	-	-	+	-	-	+
Ec-W5	+	+	-	-	+	-	-	-	-	-
Ec-B1	+	-	-	-	+	-	-	-	-	-
Ec-B2	-	-	+	-	-	-	-	-	-	-
Ec-B3	+	-	+	-	+	-	-	-	-	-
Ec-Ws1	+	-	+	+	-	-	+	+	-	-
Ec-Ws2	+	+	+	-	+	+	+	-	+	-
Ec-Ws3	-	-	-	+	+	-	+	+	-	-
Ec-U1	+	+	+	-	+	-	+	+	-	-
Ec-U2	+	-	+	-	+	-	+	-	-	+
Ec-U3	+	-	-	+	+	+	+	+	-	-
Ec-U4	+	-	-	+	-	-	-			
Ec-U5	-	-	+	+	-	+	+	-	-	+
Ec-U6	+	-	-	-	+	-	-			
Ec-U7	+	-	-	+	+	+	+	+	-	-
Ec-U8	-	-	+	-	-	-	-	-	-	-
No. of Pos.	13 (68.4)	5 (26.3)	10 (52.6)	8 (42.1)	11(57.9)	6 (31.6)	11(57.9)	6(31.6)	1 (5.3)	4 (21.1)

DISCUSSION

Pathogenic *E. coli* strains are adaptable Gram-negative bacteria that have an extensive spectrum of virulence factors, a huge region of genomic plasticity, and a complex phylogenetic structure. Infections in the urinary tract, bloodstream, and a number of other non-intestinal sites in humans are possible with the distinctively pathogenic *E. coli* in both clinical and community settings (Russo and Johnson, 2003).

In our study, pathogenic *E. coli* were obtained from the blood, wound, urine, and water samples. The occurrence of pathogenic *E. coli* in the blood samples in this investigation is consistent with the results of a study by Matsumoto *et al*. (2022) on the distribution of pathogenic *E. coli* and antibiotic resistance in blood isolates from patients in Japan. The presence of *E. coli* in the bloodstream can induce a severe host inflammatory response that can result in sepsis. Our findings revealed that *E. coli* was present in 32.0% of urine samples, which was greater than the 10% reported among UTI patients at Mulago Hospital in Kampala, Uganda (Odongo *et al*., 2020). Oftentimes, UTIs have been linked to pathogenic *E. coli* (Russo and Johnson, 2003). *E. coli* have been reported as common contaminants of wounds and isolation of *E. coli* in wound swabs in this study corroborated the findings of Makgatho *et al*. (2019), who obtained 3% *E. coli* in Limpopo Province, South Africa; 13.6% *E. coli* were obtained in Labore, Pakistan (Aizza *et al*., 2007); and 5.7 % were reported in Tamilmandu, India (Manikandan and Asmath, 2013). We found 26.3% *E. coli* in the water samples, and this comparable to the 22% reported by Taviani *et al*. (2022) in their investigation on the prevalence of waterborne pathogens and antibiotic resistance in water supply systems in Mozambique.

In our investigation, *E. coli* isolated from various clinical and water samples showed different antibiotic resistant patterns. *E. coli* were highly sensitive to Ampicillin, Gentamycin, Ofloxacin and Cotrimoxazole, with percentages of sensitivity ranging from 68.4% to 89.5%. The high sensitivity of *E. coli* to Ampicillin is consistent with the findings of Naqid *et al*. (2020) in their research on the antibiotic susceptibility pattern of *E. coli* isolated from various clinical samples in Duhok City, Iraq. This present study confirmed the findings of Kibret and Abera (2011) about the sensitivity of *E. coli* to Gentamycin. Similarly, high sensitivities of *E. coli* to Gentamycin have been reported in Southwest, Nigeria (Ochada *et al*., 2015). However, this contradicts the previous reports of Akinjogunla *et al*. (2009) and Okoli *et al*. (2002) on the high resistance of *E. coli* to Ampicillin and Gentamycin. The *E. coli* were highly resistant to Cefalexin, Nalidixic Acid, Augmentin, and Pefloxacin, and the high resistance rates to these antibiotics obtained in this study may be attributed to the prevailing use and misuse of these antibiotics in this locality.

The extracellular hydrolytic enzymes are important virulence-associated factors that enhance or contribute to the pathogenicity of organisms (Akinjogunla *et al*., 2014). In our study, 38.5%, 31.6%, and 68.4% of the *E. coli* were haemolysin-, amylase-, and gelatinase-producing strains. The percentage occurrence of haemolysin-producing *E. coli* in this study was slightly lower than the 41.9% reported by Shruthi *et al*. (2012) in their study on the virulence factors in *E. coli* isolated from patients at the Kempegowda Institute of Medical Sciences, Bangalore. However, the value we obtained for haemolysin-producing *E. coli* was higher than the 7.3% reported by Olorunmola *et al*. (2013) in Ile-Ife, Nigeria. Gelatinases are proteases secreted extracellularly by bacteria that hydrolyze or digest gelatin, a component of vertebrate connective tissue. We found more gelatinase-producing *E. coli* (68.4%) than the 19.4% reported by Shruthi *et al*. (2012) in Bangalore. The detection of amylase production in *E. coli* in this study substantiated the finding of Garba *et al*. (2021) in Gombe.

Colistin is an antibacterial agent used as a last option for the treatment of infections caused by Gram-negative bacteria, especially multidrug-resistant strains. In our investigation, 57.9% of the *E. coli* were Colistin- resistant (COR) strains. The high percentage of COR-*E. coli* in this study agrees with Singh *et al*. (2021) in their study on identification and characterization of COR-*E. coli* from the Lower Himalayan region of India, but the value was lower than the 77.1% reported in Egypt (El-Mokhtar *et al*., 2019). Colistin functions by displacing Ca2+ and Mg2+ cations that maintain the outer membrane through electrostatic interactions with the anionic phosphate groups of the lipid A moiety of lipopolysaccharide (Anjum *et al*., 2016). Of the 11 COR-*E. coli* tested in this study, 54.4%, 9.1%, and 36.4% had the COR genes mcr-1, mcr-2, and mcr-3, respectively. The percentage of COR-*E. coli* with mcr-1 was higher than the 20.8% and 23.1% detected in Assiut and Minia University Hospital, respectively (El-Mokhtar *et al*., 2019). The mcr-1 predominance in this study agrees with the findings of Newton-Foot *et al*. (2022), but this is in contrast with the reports of Deku *et al*. (2022), which reported a mcr-2 majority.

In conclusion, the *E. coli* from clinical and water samples harboured some virulence markers and were extremely resistant to Cefalexin, Nalidixic acid, Augmentin, Pefloxacin, and Colistin, indicating that these antibiotics are inappropriate for empirical treatment of *E. coli* in the study area. This study has also shown mcr-1 as the predominant mcr gene among the pathogenic *E. coli*. Thus, regular monitoring of antibiotic susceptibility in both the community and hospital settings is recommended.

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