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Title: **Antibiotic Resistance and Virulence Genes in Escherichia coli Isolated from Patients in a Tertiary Care Hospital: Implications for Clinical Management and Public Health**

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Antibiotic Resistance and Virulence Genes in *Escherichia coli* Isolated from Patients in a Tertiary Care Hospital: Implications for Clinical Management and Public Health

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ABSTRACT

Background. Diarrheal diseases, exacerbated by limited access to clean water, remain a significant global health concern. *Enterobacteriaceae*, particularly *Escherichia coli (E. coli),* are their prevalent causative agents. The emergence of antibiotic resistance poses a grave public health threat, with extended spectrum beta-lactamases (ESBL) and carbapenemases contributing significantly. This study aimed to identify the antibiogram patterns and virulence genes in *E. coli* isolates obtained from patients in a tertiary care hospital.

Method. A cross-sectional study involving 395 clinical samples from tertiary care hospital of Lahore was conducted over a period of six months. The isolation and characterization of bacterial strains were performed using culture-based, biochemical, and morphological assessments. Antibiotic susceptibility testing (AST) was carried out using the Kirby-Bauer (KB) disk diffusion method. DNA extraction and molecular identification of virulence genes were conducted through PCR. Statistical analysis was performed using Excel and SPSS.

Results. Of the 395 samples, *E. coli* was found to be the most prevalent (47.6%), followed by *Klebsiella* spp. (43.3%). AST revealed high resistance to cefuroxime (85%) and ciprofloxacin (80%). Molecular analysis identified virulence genes with traT being the most prevalent (37.2%), followed by *fimH* and *aer* (28.7%). Notably, *sfa*, *papA*, *hly*, and *cnf* genes were undetected.

Conclusion. The results showed the prevalence of antibiotic resistance genes and virulence factors in *E. coli* isolates in patients from a tertiary care hospital. The high resistance rates necessitate vigilant antimicrobial stewardship. The presence of specific virulence genes

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emphasizes the potential pathogenicity of these isolates, underscoring the importance of effective infection control measures.

Keywords: antibiotic resistance genes, antimicrobial susceptibility, *Escherichia coli*, tertiary care hospital, virulence factors

Highlights

- The study revealed alarming levels of antibiotic resistance in *E. coli* isolates.
- Investigating virulence genes in *E. coli* has given valuable insights into their potential pathogenicity.
- The findings demonstrate the widespread presence of bacterial pathogens across various departments within the healthcare settings.

1. INTRODUCTION

Diarrheal diseases affect millions worldwide, especially in low- and middle-income countries with limited access to clean and safe water [1]. People who rely on untreated water sources, such as wells, rivers, streams, and dams, are exposed to many microorganisms that may cause diarrhea, such as *Enterobacteriaceae* [2]. *Enterobacteriaceae* are common causes of diarrheal diseases and other infections in human beings and animals. These bacteria can acquire resistance to various antibiotics, making them difficult to treat and posing a severe public health threat. Among these bacteria, *Escherichia coli* or *E. coli* is one of the most important pathogens, which can also cause urinary and respiratory tract infections [3]. *E. coli* and other bacteria have evolved various mechanisms to resist the effects of antibiotics, such as blocking ion pumps, impeding antibiotic entry, developing fimbrial adhesion resistance, adhering to cell surfaces, invading hosts, colonizing tissues, and showing resistance to environmental stresses.

In 2019, six leading pathogens linked to antibiotic resistance namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were found to be responsible for over 929000 deaths, worldwide [4].

Antibiotic resistance is a significant challenge for medical treatment and public health. Some bacteria can become resistant to multiple drugs or even to all available drugs, limiting the options for effective therapy. One of the main resistance mechanisms in bacteria is the production of extended spectrum beta-lactamases (ESBL), which can inactivate anti-β-lactamides antibiotics. Carbapenems are usually considered for treating gram-negative bacterial infections, although some bacteria can also resist them by producing carbapenemases [5].

Recently, the World Health Organization (WHO) identified the widespread bacterial species that have become increasingly resistant to antibiotics, worldwide. A study found that in the absence of any measures taken to combat antibiotic resistance, mortality rates might reach almost 10 million per year by 2050. In this regard, *E. coli* has gained significant attention among all the numerous organisms that cause resistance, since it is a harmful pathogen and has a variety of virulence traits [6].

E. coli was first identified by Theodor Escherich in 1884 as a bacterium in fecal samples. It was later named after him in 1958. It is a gram-negative bacterium that typically lives in human intestines. Structurally, the genome of *E. coli* strains changes in size between extragenetic elements, pathogenic variants, and commensal strains [7].

E. coli is one of the most extensively studied microorganisms with diverse genotypes and phenotypes. Most *E. coli* strains have been isolated and identified by their O antigen (lipopolysaccharide), H antigen (flagellar), and K antigen (capsular). Pathogenic strains of *E. coli* can be classified into intestinal and extraintestinal types, depending on their site of infection (Figure 1). These strains can be differentiated by their colony characteristics and biochemical reactions on various culture media, such as blood agar, MacConkey agar, Eosin-methylene blue agar (EMB), and nutrient agar [8].

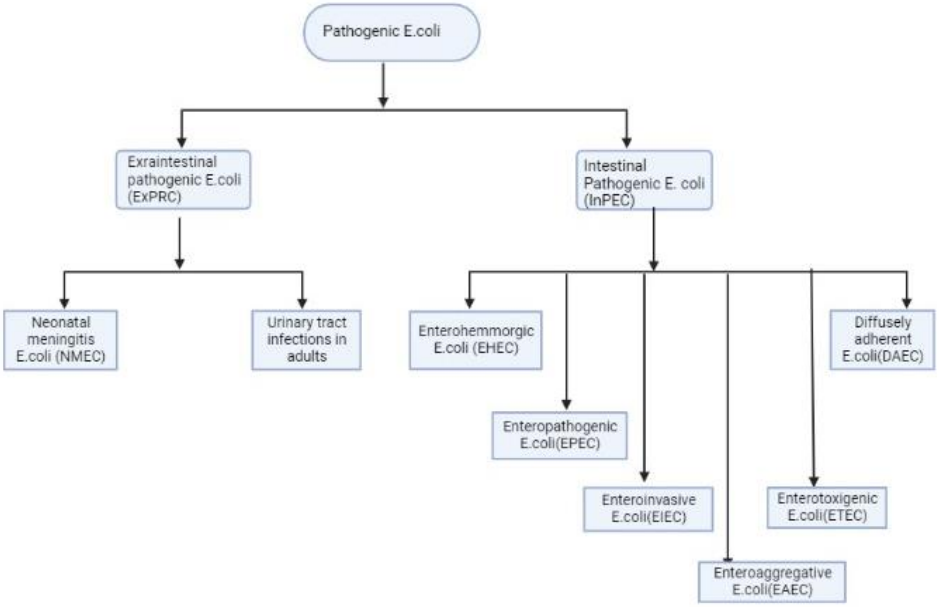

Figure 1. Schematic Illustration of Pathogenic *E. coli*

The treatment of *E. coli* infections depends on the type and severity of the disease, as well as the resistance patterns of the bacteria. For lower urinary tract infections (UTIs), oral antibiotics, such as trimethoprim-sulfamethoxazole or nitrofurantoin, are usually prescribed. While, for pyelonephritis, intravenous antibiotics, such as ciprofloxacin or ceftriaxone, are used. Antibiotic therapy is not recommended for diarrheal diseases caused by *E. coli,* unless there are complications or systemic symptoms. Instead, symptomatic treatment with loperamide and trimethoprim-sulphamethoxazole can be given. However, antimotility drugs should be avoided for infections caused by *E. coli* O157 strain, as they can increase the risk of hemolytic urinary syndrome (HUS). To prevent *E. coli* infections, passive immunization and hygiene measures can be considered. Some studies have also suggested that cranberry juice, which contains flavonoids that can

prevent the attachment of pili to the bladder epithelium, can be a natural alternative to urine acidification [9].

A significant challenge in treating *E. coli* infections is the emergence of multi-drug resistant (MDR) bacteria, which can reduce the effectiveness of antibiotics. Numerous other factors contribute to its spread, although the abuse or overuse of antibiotics is the main factor in the emergence of AMR [10].

Moreover, the development of new antibiotics is slow and insufficient to cope with the problem. Gram-negative bacteria, such as *E. coli,* have an outer membrane that acts as a permeability barrier, limiting the entry of many antibiotics. The bacteria can also alter the target structures of antibiotics, thus preventing their binding and action. Multiplex PCR is a valuable technique for gene amplification and the detection of virulence-associated genes, which can help to distinguish different pathotypes of *E. coli* [11].

E. coli is typically regarded as a commensal of the gastrointestinal tract. However, these commensal bacteria can transform into a highly adapted pathogen through random point mutations or via the acquisition of virulence proteins, both chromosomal and extra-chromosomal [12].

E. coli can produce various virulence factors contributing to its pathogenicity and ability to cause different diseases. One of these factors is *hly* (hemolysin), a toxin that causes cell lysis and tissue damage, leading to vacuolization, biofilm formation, and vesicle creation in the host cell [13–15]. Another is *cnf* (cytotoxic necrotizing factor), a toxin that causes cellular damage and tissue destruction, thus enhancing the invasiveness of the bacterium. Then, there are *PapG2* and *papA* genes encoding P fimbriae, which mediate the adhesion of the bacteria to various surfaces and cells, facilitating colonization, motility, biofilm formation, and intracellular survival [5,16]. Similarly, *PapG1*, *Prsj96*, *Pap1*, *FimH*, *Sfa*, *Afa*, and *aer* (aerobactin) genes encode other adhesions or iron acquisition factors, which also promote adhesion, colonization, and survival of the bacteria within the host [17–19].

In addition to adhesion, the genes *feoB*, *fyuA*, and *traT* are involved in iron acquisition systems. These systems are essential for the bacteria to obtain vital nutrients including iron and manganese from the host. They also play a role in adherence to cell surfaces, host invasion, colonization, and the expression of other virulence genes. This persistence within the host, along with the resistance to environmental stresses, further solidifies the bacteria's ability to cause infections [20, 21].

Lastly, protectins like *TraT* shield against serum bactericidal activity and opsonization, enhancing the bacterium's ability to evade the host's immune responses. They also contribute to adherence to cell surface, host invasion, colonization, and propagation, collectively bolstering the bacterium's pathogenic potential [22]. *E. coli* can also produce factors that help it acquire iron and other essential nutrients from the host and protect itself from the host’s immune system. Some of these factors are *feoB*, *FyuA*, and *TraT* genes encoding iron acquisition systems, which enable the bacteria to scavenge iron and manganese from the host. They also influence the adherence, invasion, colonization, and expression of other virulence genes of the bacteria [23–25]. *TraT*, a gene encoding a protecting factor, shields the bacteria from serum bactericidal activity and opsonization, allowing them to escape the host’s immune responses. It also

affects bacteria's adherence, invasion, colonization, and propagation (Table 1).

Table 1. Genes and their Role in Pathogenesis

Genes	Virulence Factors	Role in Pathogenesis	Reference
hly (hemolysin)	Toxins	Destruction of cell and tissue damage causes vacuolization in the host cell, settlement, formation of biofilm, and vesicle formation.	[14–26]
cnf (cytotoxic necrotizing factor)			
papG1 (P fimbriae)	Adhesion	Adherence to other surfaces or cells, colonization, mediate motility, formation of biofilm, and survival inside the cell.	[14, 26, 27]
papA (P fimbriae)			
papG2 (P fimbriae)			
papG3 (P fimbriae)			
Pap1			
FimH			
Sfa			
Afa			
aer (aerobactin)	Iron acquisition systems	Take iron and manganese from the host, adherence to the surface of the cell, invade the host, colonization, and express virulence genes, persistence in the host, and resistance to environmental stresses.	[14, 19, 26, 27)
feoB			
FyuA			
TraT	Protectins	Protect from serum bactericidal activity and opsonization, adherence to the cell's surface, invasion of the host, colonization, and propagation.	[14]

2. METHODOLOGY

A cross-sectional study was conducted over a period of six months, from January 2023 to June 2023. A total of 395 clinical samples were collected from various wards of a tertiary care hospital of Lahore, Pakistan. Various samples were processed including urine, blood, sputum, swab, and pus samples. These samples were subsequently transported to the microbiology laboratory.

2.1. Isolation and Characterization of Bacterial Strains

The identification of bacteria involved a multi-step process encompassing their cultural, biochemical, and morphological assessments. Culture-based identification was carried out using blood agar, MacConkey agar, nutrient agar, and cystine lactose electrolyte deficient (CLED) agar, with CLED being specifically employed for urine cultures. Blood agar, MacConkey

agar, and nutrient agar were utilized for specimens other than urine. Following inoculation, the culture plates were incubated at 37℃ for 18-24 hours. Afterwards, interpretations were made. Biochemical identification was conducted on triple sugar iron (TSI) agar, Simmons citrate agar, and sulfide indole motility (SIM) agar. Morphological identification was accomplished through Gram staining, wherein gram-positive bacteria exhibited a purple hue, while gram-negative bacteria displayed a pinkish color under microscopic examination.

2.2. Antibiotic Susceptibility Testing (AST)

Pure colonies of *E. coli* were subjected to susceptibility and resistance testing against various classes of antibiotics. This assessment was performed using Muller-Minton agar via the Kirby-Bauer (KB) disk diffusion method. The McFarland standard of turbidity (0.5) was employed. Suspensions of each isolate were prepared and applied to the MH plate. Antibiotic disks were impregnated on MH agar plates and placed in the incubator for 18-24 hours at 37℃. Interpretations were made by measuring the zones of inhibition. Following CLSI guidelines, the results were categorized as sensitive (S), resistant (R), or intermediate sensitive (IS). A total of seven (07) antibiotic disks were employed in this study including ciprofloxacin (CIP), levofloxacin (LEV), cefuroxime (CXM), cefoxitin (FOX), cefixime (CFM), cefepime (FEP), and tazobactam (TZP).

2.3. DNA Extraction

DNA extraction followed the manufacturer's protocol utilizing the Geneaid Nucleic Acid Extraction Kit II which included lysis, AD, Wash I, Wash buffer II, and normal saline. To verify the nucleic acid extraction, agarose gel electrophoresis was employed. Extracted DNA was stored at -20℃.

2.4. Molecular Identification

Polymerase chain reaction (PCR) was employed to amplify the targeted DNA after its successful extraction. The thermal cycler utilized was the geneamp® PCR Systems 9700 by the company. A commercially prepared master mix was used, and primers were designed by extracting the gene of interest from the NCBI database, with primer design and ordering conducted through the primer3 website (Table 2). The 14 genes identified and selected for this study were *traT, fimH, aer, feo, papGIA2, fyuA, pap1, afa, papG96, prsJ96, sfa, papA, hly,* and *cnfs* (Table 2). The PCR product was subjected to agarose gel electrophoresis and amplification was confirmed by exposing the gel to a UV illuminator.

Table 2. Sequence for the List of Primers for the Virulent Strains of *E. coli*

Sr. No	Gene	Primer	Sequence (5'---3')	PCR Product Size (KBp)	Annealing Temperature (℃)	Reference
1	*fimH*	*fimH-F*	AACAGCGATGATTTCC AGTTTGTGTG	465	65	[25]
		fimH-R	ATTGCGTACCAGCATT AGCAATGTCC			
2	*papC*	*Pap1 F*	GACGGCTGTACTGCAG GGTGTGGCG	328	65	[25]
		Pap1 R	ATATCCTTTCTGCAGG GATGCAATA			

Sr. No	Gene	Primer	Sequence (5'---3')	PCR Product Size (KBp)	Annealing Temperature (°C)	Reference
3	*cnf*	*Cnfs F*	TTATATAGTCGTCAAG ATGGA	693	58	[13, 25]
		Cnfs R	CACTAAGCTTTACAAT ATTGA			
4	*afa*	*afa F*	GCTGGGCATCAAACTG ATAACTCTC	750	65	[4]
		afa R	CATCAAGCTGTTTGTT CGTCCGCCG			
5	*hly*	*hly F*	AACAAGGATAAGCAC TGTTCTGCT	1177	63	[4]
		hly R	ACCATATAAGCGGTCA TTCCCGTCA			
6	*Sfa*	*sfa F*	CGGAGGAGTAATTAC AAACCTGGCA	410	65	[4]
		sfa R	GAGAACTGCCCGGGT GCATACTCT			
7	*papG2*	*papGIA2F*	GGGATGAGCGGGCCT TTGAT	190	72	[17]
		papGIA2R	CGGGCCCCCAAGTAA CTCG			
8	*papG3*	*prsJ96 F*	GGCCTGCAATGGATTT ACCTGG	258	72	[17]
		prsJ96 R	CCACCAAATGACCATG CCAGAC			
9	*aer*	*Aer F*	TACCGGATTGTCATAT GCAGACCGT	602	61	[12]
		Aer R	AATATCTTCCTCCAGT CCGGAGAAG			
10	*fyuA*	*fyuA F*	GTAAACAATCTTCCCG CTCGGCAT	850	63	[24]
		fyuA R	TGACGATTAACGAACC GGAAGGGA			
11	*papG1*	*papGJ96 F*	TCGTGCTGAGGTCCGG AATTT	461	72	[17]
		papGJ96 R	TGGCATCCCCCAACAT TATCG			
12	*feoB*	*feoB-F*	AGCTGGCGACCTGATA GAACAATG	470	63	[16]
		feoB-R	AATTGGCGTGCATGAA GATAACTG			
13	*papA*	*papA-F*	ATGGCAGTGGTGTTTT GGTG	720	63	[16]
		papA-R	CGTCCCACCATACGTG CTCTTC			
14	*TraT*	*TraT F*	GGTGTGGTGCGATGA GCACAG	290	63	[19]
		TraT R	CACGGTTCAGCGATCC CTGAG			

2.5. Statistical Analysis

Statistical analysis was performed using Excel and Statistical Package for Social Science (SPSS) software. Chi-square test was performed and *p*-value less

than 0.05 was considered as statistically significant.

3. RESULTS

Table 3. Demographics

Variables	Parameter	Percentage
Gender	Female	29
	Male	159
Age Groups (Years)	<10	5
	11-20	1
	21-30	48
	31-40	81
	41-50	32
	51-60	17
	61-70	4
	71-80	0
Wards	Medical	82
	Surgical	6
	Outpatient Department	41
	Emergency	19
	Nephrology	40
Clinical Sample	Urine	35.9
	Body fluid	22
	Swab	26.3
	Pus	2.5
	Blood	13.2
Isolates	*E. coli*	47.6
	Klebsiella spp.	43.3
	Citrobacter spp.	1.3
	Acinetobacter spp.	3.5
	Moragnella spp.	0.8
	Proteus spp.	1.5
	Enterobacter spp.	0.5
	Serratia spp.	1.5

3.1. Patients' Demographics

The study gathered and analyzed information about demographic variables including gender and age groups. Among the participants, there were 29 women (*n*=29; 15.5%) and 159 men (*n*=159; 84.5%). In terms of age-wise distribution, there were 5 cases in the 1-10 years age category (*n*=5; 2.7%), 1 case in the 11-20 years age group (*n*=1; 0.5%), 48 cases in the 21-30 years age group (*n*=48; 25.6%), 81 cases in the 31-40 years age group (*n*=81; 43.1%), 32 cases in the 41-50 years age group (*n*=32; 17%), 17 cases in the 51-60 years age group (*n*=17; 9.1%), 4 cases in the 61-70 years age group (*n*=4; 2.1%), and no cases in the 71-80 years age group (n=0; 0%). These findings provide valuable insights into the distribution of participants based on gender and age (Table 3).

3.2. Ward-wise Sample Distribution

The study examined the distribution of various bacterial organisms across different departments or units within a medical facility. *E. coli* was found to be the most prevalent organism, with 82 cases identified in the medical department (*n*=82; 20.8%), 6 cases in the surgical department (*n*=6; 1.5%), 41 cases in the OPD (*n*=41; 10.4%), 19 cases in the emergency (*n*=19; 4.8%), and 40 cases in the nephrology department (*n*=40; 10.1%). Further, *Klebsiella* species closely followed with 87 cases in the medical department (*n*=87; 20.7%), 7 cases in the surgical department (*n*=7; 1.7%), 25 cases in the OPD (*n*=25; 6%), 14 cases in the emergency (*n*=14; 3.3%), and 38 cases in the nephrology department (*n*=38; 9%).

Citrobacter species were less common, with 3 cases found in the medical department (*n*=3; 60%), 1 case in the OPD (*n*=1; 20%), and 1 case in the emergency (*n*=1; 20%). Whereas *Acinetobacter* species were identified in 4 cases in the medical department (*n*=4; 20%), in 2 cases in the surgical department (*n*=2; 10%), in 4 cases in the OPD (*n*=4; 20%), and in 4 cases in the nephrology department (*n*=4; 20%).

Moragnella species were isolated in 1 case in the medical department (*n*=1;

33.3%) and in 2 cases in the nephrology department (*n*=2; 66.7%). *Proteus* species were found in 3 cases in the medical department (*n*=3; 50%), in 1 case in the emergency (*n*=1; 16.7%), and in 2 cases in the nephrology department (*n*=2; 33.3%). *Enterobacter* species were exclusively identified in the medical department (*n*=2; 100%). *Serretia* species were found in 3 cases in the medical department (*n*=3; 50%) and in 3 cases in the nephrology department (*n*=3; 50%). These findings provide valuable insights into the distribution of bacterial organisms across different units within the medical facility (Table 3).

3.3. Sample Isolation Statistics

In the current study, various bacterial organisms were isolated from different specimens. *E. coli* was found to be the most prevalent, with 188 cases identified. These were distributed across urine samples (*n*=63; 33.5%), body fluids (*n*=47; 25%), swabs (*n*=45; 23.9%), pus samples (*n*=6; 3.2%), and blood samples (*n*=27; 14.4%). *Klebsiella* species followed closely behind with 171 isolates. These were distributed across urine samples (*n*=62; 36.3%), body fluids (*n*=34; 19.9%), swabs (*n*=52; 30.4%), pus samples (*n*=4; 2.3%), and blood samples (*n*=19; 11.1%). *Citrobacter* species were less frequently encountered, with a total of 5 cases only. These were primarily found in urine (*n*=3; 60%) and body fluid samples (*n*=1; 20%). *Acinetobacter* species were identified in 14 cases, predominantly in urine (*n*=8; 57.1%) and body fluids (*n*=3; 21.4%). *Moragnella* species were isolated in only 2 cases, with a single case each among body fluids (*n*=1; 33.3%) and swabs (*n*=1; 33.3%). *Proteus* species were found in 6 cases, mostly in urine (*n*=3; 50%) and swab specimens (*n*=1; 16.7%). *Enterobacter* species were found to be the least prevalent. They were identified only in 2 cases among swab samples (*n*=1; 50%). Finally, *Serretia* species were isolated in 6 cases distributed across urine (*n*=3; 50%), body fluids (*n*=1; 16.7%), and swabs (*n*=2; 33.3%). A total of 395 bacterial isolates were documented across all specimen types in this study (Table 3).

3.4. Antimicrobial Susceptibility Test (AST) Results

The AST results revealed that the isolates displayed highest resistance rates against cefuroxime, exhibiting a resistance rate of 85%. The results also revealed significant variations in the resistance and sensitivity percentages of various antibiotics. Ciprofloxacin exhibited a high resistance rate of 80% with only 20% sensitivity. Levofloxacin also showed notable resistance at 70%. Consequently, it showed a slightly higher sensitivity at 30%. Tazobactam showed a lower resistance rate of 55%, thus indicating a higher sensitivity of 45%. Cefixime and cefepime exhibited similar resistance rates at 65%, with corresponding sensitivity rates of 35%. Cefoxitin demonstrated a relative resistance rate of 40%, hence indicating a higher sensitivity at 60%. However, cefuroxime displayed the highest resistance rate among the antibiotics tested at 85% and the lowest sensitivity rate at 15%. These findings highlight the varying effectiveness of these antibiotics against the tested strains, providing crucial information for clinical decision-making and treatment strategies (Figure 2).

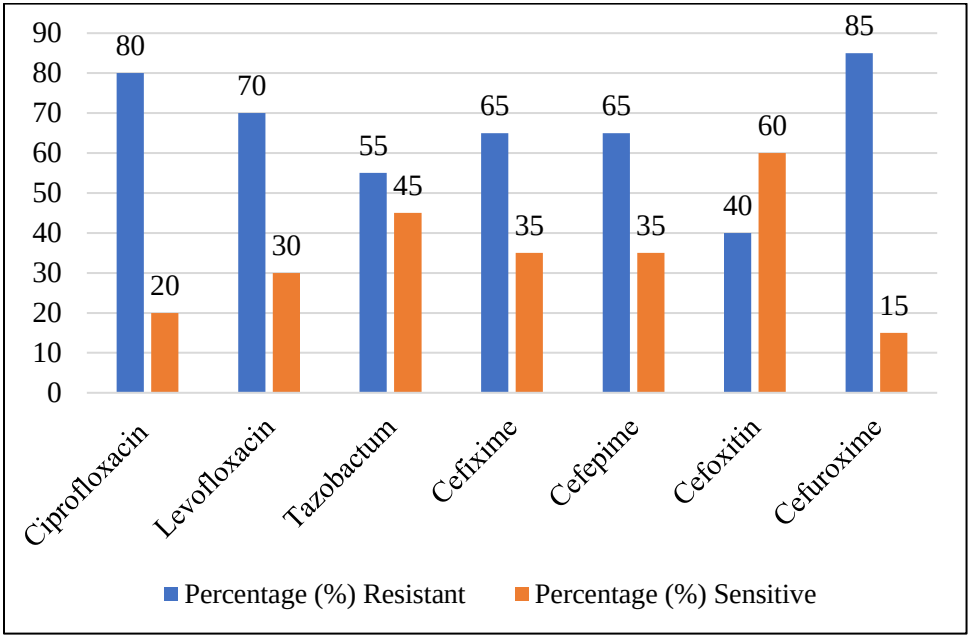

Figure 2. Antimicrobial Susceptibility Pattern

3.5. Virulence Gene Detection

Out of the total 14 genes, the virulence gene exhibited varying prevalence percentages. The *traT* gene was the most prevalent, since it was identified in 37.2% of the isolates (n=70). Following closely, both *fimH* and *aer* genes were present in 28.7% of the samples (n=54). The *feoB* gene was observed in 24.4% of the isolates (n=46), while *papGIA2* was identified in 20.7% of cases (n=39). Additionally, *fyuA* was detected in 18% (n=34), *pap1* in 13.8% (n=26), and *afa* in 10.6% (n=20) of the isolates. Less frequently observed was *papG96*, found in 5.3% of samples (n=10), while *prsJ96* was found in 1% (n=2) of samples. Moreover, *sfa*, *papA*, *hly*, and *cnf* were not detected in any isolates (Figure 3).

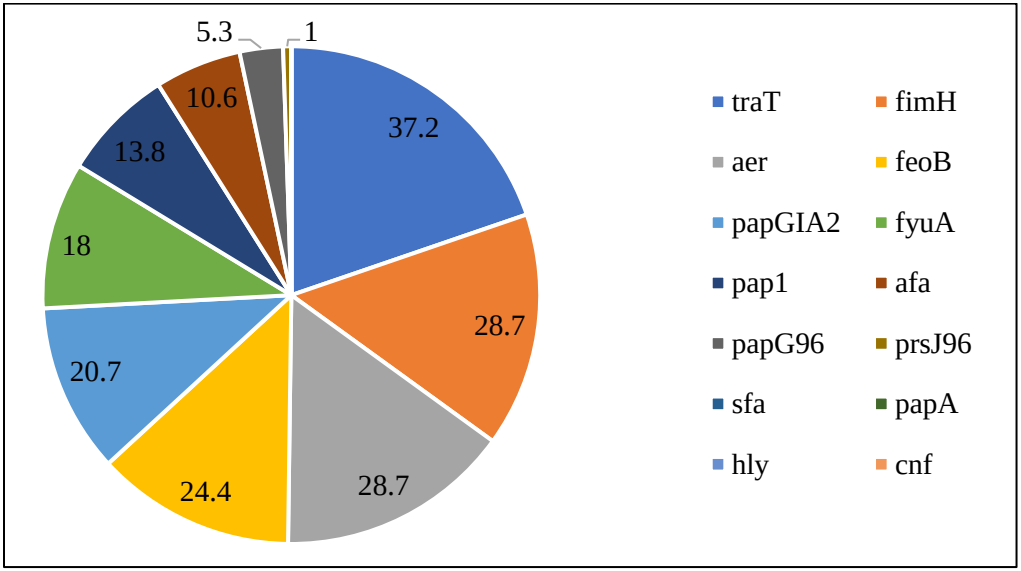

Figure 3. Proportion of Virulence Genes

4. DISCUSSION

The current study focused on the molecular identification of antibiotic-resistance genes in *E. coli* isolated from patients in a tertiary care hospital in Lahore, Pakistan. The findings are critical in understanding the prevalence of antibiotic resistance and the distribution of virulence genes in clinical isolates. The results indicate a high prevalence of *E. coli*, with 82 cases identified in the medical department alone. This aligns with the previous research highlighting *E. coli* as a major pathogen responsible for various infections, including urinary tract infections (UTIs) and diarrheal diseases [5]. *Klebsiella* species were also found to be prevalent, emphasizing the importance of understanding resistance patterns in multiple pathogens [16, 18, 20]. Furthermore, AST results revealed concerning levels of resistance, particularly against cefuroxime and ciprofloxacin, with rates of 85% and 80%, respectively. This high resistance level underscores the urgent need for continued surveillance and development of alternative treatment strategies. It was identified that 47.6% of *E. coli* isolates were resistant to cefuroxime and ciprofloxacin. According to recent European studies in pediatric patients with a UTI, the rate of *E. coli* resistant to cefuroxime ranges from 14.5% in Germany to 19% in Ukraine [15]. The lower resistance rates observed for tazobactam and cefoxitin suggest their potential as treatment options, although they may not be universally effective [21, 23, 26]. Identifying various virulence genes in *E. coli* isolates provides valuable insights into these strains' potential pathogenicity and invasiveness. The presence of genes such as *traT*, *fimH*, and *aer* suggests an enhanced ability to adhere to host cells and resist immune responses, potentially contributing to their pathogenicity [5]. The absence of genes including *hly* and *cnf* may indicate a specific strain profile or population in this clinical setting [27, 28].

This study scrutinized 188 pathogenic *E. coli* isolates for various virulence genes. These genes play a crucial role in the pathogenicity of *E. coli* strains. *TraT* displayed the highest prevalence among the genes examined, being identified in 37.2% of the isolates (*n*=70) [29]. *TraT* is associated with resistance against the host immune system and remains a crucial factor in colonization. Following closely, both *fimH* and *aer* genes were present in 28.7% of the samples (*n*=54). Most studies reported that the most frequent genes in UPEC strains are *fimH* (68% to 96%) and *aer* (47% to 66%). Notably, the variation in the frequency of these genes is due to strains belonging to different phylogenetic groups [30]. A study reported that among UPEC strains, *fimH*, *sfa*, *hlyA*, and *fyuA* genes were more frequent in the B2 phylogenetic group, as compared to group D [9]. However, phylogenetic classification of the strains was not performed in this study.

FimH is known for its role in adherence to host cells, a critical step in establishing infections. Similarly, air contributes to bacterial motility and host colonization. The *feoB* gene was observed in 24.4% of the isolates (*n*=46). *FeoB* is involved in iron acquisition, an essential nutrient for bacterial growth and virulence. Additionally, *papGIA2* was identified in 20.7% of cases (*n*=39). This gene is associated with adherence to uroepithelial cells, a hallmark of uropathogenic *E. coli*. *FyuA*, detected in 18% of the isolates (*n*=34), is also involved in iron acquisition. *Pap1* and *afa* were found in 13.8% (*n*=26) and 10.6% (*n*=20) of cases, respectively. These genes are also associated with adherence to host cells. Less frequently observed were *papG96* found in 5.3% of samples (*n*=10) and *prsJ96* found in 1% (*n*=2) of samples, while *sfa*, *papA*, *hly*, and *cnf* were not detected. This detailed genetic analysis sheds light on the prevalence of specific virulence genes within the tested *E. coli* isolates, offering valuable insights into their potential to cause infection [31–34].

4.1. Implications

This study has important implications for clinical practice and public health. The high prevalence of antibiotic resistance highlights the critical need for effective stewardship programs and the development of new treatment strategies. Additionally, understanding the distribution of virulence genes provides insight into the severity and invasiveness of these strains, which is crucial for patient management.

4.2. Limitations

While this study provides valuable information, it also has some limitations. The study's cross-sectional design provides a snapshot of resistance patterns at a specific point in time. Whereas long-term trends may vary. Additionally, the study focused on isolates from a single tertiary care hospital, which may not represent regional or national trends. In the future, conducting longitudinal studies intended to track changes in resistance patterns over time would be beneficial. Expanding the current study to include a broader range of healthcare facilities and regions would provide a more comprehensive understanding of resistance profiles in different settings.

4.3. Conclusion

This study shed light on the prevalence of antibiotic resistance and the distribution of virulence genes in *E. coli* isolates from patients in a tertiary care hospital. The high levels of resistance observed underscore the urgent need for continued research and development of effective treatment strategies. Additionally, understanding the

genetic makeup of these strains is crucial for informed clinical decision-making and patient management.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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