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Original Research Paper

Investigation resistance and virulence of *E. coli* in local pathogenic samples

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Abstract: *Escherichia coli* is a common opportunistic pathogen responsible for a wide range of infections. The emergence of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing strains poses a major challenge to treatment. **Objective**: This study aimed to determine the antibiotic resistance patterns, motility, ESBL production, and distribution of virulence and resistance genes (*blaTEM*, *Pap*, and *ompT*) among *E. coli* isolates from different clinical sources. **Methodology**: A total of 39 *E. coli* isolates were recovered from urine, blood, wound, and stool samples. Identification was performed using the Vitek 2 system, Gram staining, and biochemical tests. Antimicrobial susceptibility testing was conducted using standard methods. ESBL production was detected by the double-disk synergy test (DDST), motility was assessed using semi-solid medium, and gene detection was carried out by conventional PCR. **Results**: Most isolates were obtained from urine (66.67%). High resistance was observed to amoxicillin-clavulanate (97.44%) and ceftoxime (82.05%), whereas low resistance was seen to meropenem (7.6%). ESBL production was confirmed in 79.49% of isolates. The *ompT* gene was the most prevalent, followed by *blaTEM* and *Pap*. **Conclusion**: The high frequency of MDR and ESBL-producing *E. coli* underscores the urgent need for continuous surveillance, rational antibiotic use, and molecular monitoring to control resistance spread.

Keywords: *Escherichia coli***,** *OmpT***,** *Pap***,** *blaTEM* genes

1. Introduction

Escherichia coli is widespread around the world and can be found everywhere. It is also part of the gut micro biota in animals and human and is one of the most important members of the *Enterobacteriaceae* family. It is naturally present (normal flora) in the digestive tract, specifically the colon. and rarely causes disease. However, it can adapt to become pathogenic after acquiring virulence factors. [1,2] *E. coli* causes meningitis, diarrhea, and sepsis and also urinary tract infections (UPEC), being responsible for about 90% of urinary tract infections worldwide. Infections are about 14 times more common in females than in males [3].

E.coli possesses several virulence factors, which are responsible for its pathogenicity. Among the most important virulence factors are the presence of iron chelates (siderophores), cytotoxic necrotizing factor, and virulence factors cell associated with the bacterial surface. These include a number of different types of adhesive organelles that promote its attachment to host tissues, such as capsules, flagella, and lipopolysaccharides, which provide protection, motility, and toxicity to *E.coli* [4] each *E.coli* pathogenesis pattern

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has a distinct pathogenicity mechanism and profile, encoded by specific gene clusters. Pathogenicity associated genes may encode activities such as adhesion, invasion, and binding invasion, attachment, motility, and toxin activity [5, 6].*E.coli* are characterized by their resistance to antibiotics, exhibiting several patterns such as: extensively drug-resistant (MDR), broadly drug-resistant (XDR) [7]. Their high resistance to antibiotics is due to the presence of resistance enzymes such as beta-lactamases, which confer resistance to beta-lactam antibiotics, and enzymes that confer resistance to quinolones and aminoglycosides. *E. coli* also possesses other mechanisms that enable it to resist antibiotics, such as reducing drug absorption, inactivating the drug, altering the drug target site, and efflux pumps [8-10] Antibiotics are frequently used to treat human infections as well as to prevent illness. Numerous studies indicate that incorrect antibiotic selection and misuse may cause resistance in a variety of bacteria and complicate the treatment of bacterial diseases. The development of resistance to the majority of first-line antimicrobial drugs has made treating *E. coli* infections more difficult [11].This study aimed to evaluate the antibiotic resistance patterns, motility, and extended-spectrum β-lactamase (ESBL) production of *Escherichia coli* isolates recovered from different clinical samples. It also sought to investigate the molecular distribution of selected virulence and resistance genes (*blaTEM*, *Pap*, and *ompT*) using conventional PCR and understand the relationship between phenotypic resistance and the presence of virulence determinants.

2. Methodology

Samples collection

One handered fifty samples from different clinical sources . Samples were collected from Ramadi Teaching Hospital, Fallujah Teaching Hospital, Fallujah Women's and Children's between August1 and November 15,2025. Isolates were cultured on MacConkey agar ,EMB agar, CHROM agar. The Gram stain, triple sugar fermentation test, citrate test, urease test, indole test, Voges Proskauer, and methyl red test and Vitek_2 system.were used to confirm growths suspected of being *E. coli.*

Antibiotic Susceptibility Test

Disc diffusion method was used to examine the antibiotic sensitivity pattern of *E. coli* isolates on Muller Hinton agar plates. Ten antibiotic disks tested were, cefotaxime, gentamicin, levofloxacin, nalidixic acid and meropenem, amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin ,norfloxacin, chloramphenicol . After incubation at 37°C for 24 hour, 39 Isolates were declared as sensitive or resistant on the basis of zone of inhibition following the criteria of Clinical Laboratory standards Institute [12].

Detection of Extended Spectrum β–Lactamases

Thirty nine isolates were taken from E.coli to perform the investigation of βeta-lactamase by double-disk synergy, where the bacterial suspension of E.coli bacteria was present at the age of 18-24 hours, where its density was compared with the solution of the standard constant turbidity McFarland, whose cell number is approximately equal to 1.5×108. Mueller-Hinton medium was inoculated by using a sterile cotton swab in the method of brushes (mat method) and left to dry for 10 minutes. After that an antibiotic disc of amoxicillin/clavulanic acid (20/10mg was positioned in the center of the petri dish, while the antibiotic discs of ceftriaxone (30mg) aztreonam (30mg), ceftazidime (30mg), cefotaxime (30mg), and were arranged around the amoxicillin/clavulanic acid , ensuring a 20mm distance between each. The zones of inhibition were then examined, revealing that each of the four discs exhibited an increased inhibition zone towards the central amoxicillin/clavulanic acid, indicating a positive result for the production of ESBL[13].

Table1: Primers sequence used for the detection of *Escherichia coli*

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Gene	Gene	Sequence of primer (5'-3')	Tm.	Product size (bp)	Ref.
OmpT	Forward	ATCTAGCCGAAGAAGGAGGC	60	559	15
	Reverse	CCCGGGTCATAGTGTTCATC			
Pap	Forward	AGGGGCAGGGTAAAGTAACT	61	250	Primer design
	Reverse	TGTCCATTAACTATCGGGCC			
blaTEM	Forward	ATAAAATTCTTGAAGACGAAA	55	1080	16
	Reverse	GACAGTTACCAATGCTTAATC			

Motility Test

A bacterial colony was placed into tubes containing motility test media by stabbing them down to a depth of 3 cm, being careful not to touch the bottom of the tubes. After that, the tubes were incubated at 37 °C for a full day. A positive outcome is indicated by the cloudy forms surrounding the stabbing site [14].

Gene detection by Conventional PCR

Conventional polymerase chain reaction (PCR), a molecular method for identifying particular genetic markers in bacterial isolates, was employed to detect virulence genes such OmpT, blaTEM, Pap, and in 16 isolates. This method allows for the validation of the presence or absence of specific virulence factors by amplifying target DNA sequences using gene-specific primers. All of the samples had their genomic DNA extracted and used in amplification procedures. The existence of these genes was evaluated using certain primers. Agarose gel electrophoresis was used to examine the PCR results after the amplification process was carried out under ideal conditions.

DNA extraction

The ELKTM procedure (ELK, Chin) was used to extract genomic DNA. A Quantus Fluorometer was used to quantify the purified DNAs, and their concentration and purity were assessed for subsequent molecular investigations.

Preparation of primers

Macrogen Company provided the primers in lyophilized form. Nuclease-free water was used to dissolve lyophilized primers, resulting in a stock solution with a final concentration of 100 pmol/µl. 90µl of nuclease-free water was mixed with 10µl of primer stock solution (stored at freezer -20 C) to create a working primer solution of 10pmol/µl Table (1)(2)

Table 2: Conditions of PCR analyzed by agarose gel electrophoresis

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:15	40
Annealing	59-63	00:15	
Extension	72	00:15	
Final Extension	72	10:00	1

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (version 9.5.1, LLA, CA, USA) used for statistical analysis. The parametric analysis was performed Chi-square (and Fisher's exact) and ordinary two-way ANOVA tests for comparsion between different groups.

3. Results and discussion

A total of 39 clinical isolates of E.coli were recovered from the various clinical specimens of patient. The highest number of isolates were 26 (66.67%) obtained from the urine samples followed by blood 5(12.82%) ,stool and wound swab 4 (10.26%),These isolates were identified using the Vitek 2 system, their microscopic characteristics were determined through Gram staining

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as it is gram-negative bacteria, and their culture characteristics and biochemical test figure 1.

Fig 1: *E.coli* isolates based on their cultural features and characteristics

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing results revealed that more isolates were resistant to amoxicillin-clavulanate resistance rate (97.44%). Ceftoxime (82.05%), ciprofloxacin and nalidixic acid (74.36%)%),levofloxacin (56.41%)and Norfloxacin (46.15%) Gentamicin and Piperacillin -tazobactam (30.77%). In contrast, the low resistance rates were observed for Chloramphenicol (10.26%)and Meropenem (7.6%) figure2, table 3.

Fig 2: variable susceptibility of *E.coli* isolates to the tested antibiotic

Table 3: Susceptibility of *E.coli* isolates to the tested antibiotics

Name antibiotics	R(%)	I(%)	S(%)
Meropenem	3(7.6%)	1(2.56%)	35(89.74 %)
Amoxillin- clavulnate	38(97.44 %)	1(2.56%)	0(0.0%)
Piperacillin -tazobactam	12(30.77 %)	2(5.13%)	25(64.10 %)
Ceftoxime	32(82.05 %)	0(0.0%)	7(17.95 %)
levofloxacin	22(56.41 %)	12(30.77 %)	5(12.82 %)
Ciprofloaxacin	29(74.36 %)	3(7.69%)	7(17.95 %)
Nalidixic acid	29(74.36 %)	2(5.13%)	8(50.51 %)
Norfloxacin	18(46.15 %)	2(5.13%)	19(48.72 %)
Chloramphenicol	4(10.26 %)	1(2.56%)	34(87.18 %)
Gentamicin	12(30.77 %)	2(5.13%)	25(64.10 %)

Detection of Extended Spectrum β–Lactamases

The double disk synergy test (DDST) was used to evaluate the isolates for ESBL enzyme production (Figure3). According to the results table 4, 31 isolates (79.49%) produced ESBL, whereas 8 isolates (20.51%) did not produce the enzyme.

Table 4: Percentage of ESBL Producers Among the Total *E.coli* Isolates (n=39)

Subject	isolate number	percentage
ESBL-Production	31	79.49%
ESBL-non Production	8	20.51%
total	39	100%

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Fig. 3: Shows production ESBL enzyme

Motility

Motility of the isolates was tested using semi-solid medium and it was found that 90% of the isolates were motile figure 4.

Fig.4: Motility Test of *Escherichia coli* Isolates in Semi-Solid Medium

Molecular detection of blaTEM, Pap, OmpT genes

Results revealed variable distribution of the *blaTEM*, *Pap*, and *OmpT* genes among *E. coli* isolates from different clinical sources. The *OmpT* gene showed the highest prevalence, being detected in all isolates from urine and wound samples (100%) and in 50% of blood isolates, while it appeared in only 25% of stool isolates. The *blaTEM* gene was most frequent in wound isolates (75%) and moderately present in urine and blood isolates (50%), but absent in stool isolates, The *Pap* gene was detected in half of the urine and blood isolates (50%) and only in 25% of wound isolates table5, figure5,6,7.

Table 5: Prevalence of Virulence and Resistance Genes in *E.coli* Isolates of clinical Samples

Type sample	***ompT***	***Pap***	***blaTEM***
Urine(4)	4(100%)	2(50%)	2(50%)
Stool(4)	1(25%)	0(0%)	0(0%)
Wound(4)	4(100%)	1(25%)	3(75%)
Blood(4)	2(50%)	2(50%)	2(50%)

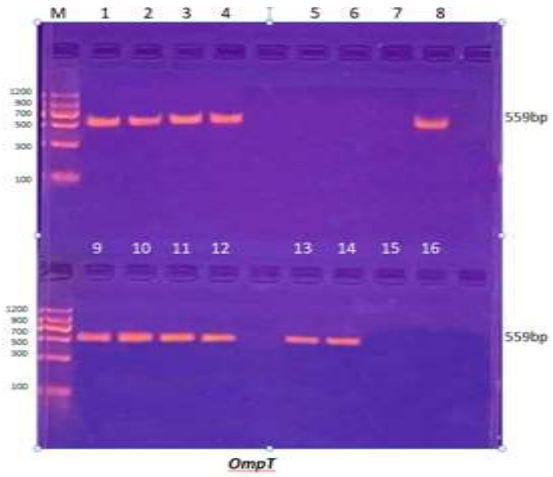

Fig 5: Gel Electrophoresis of PCR Product to *OmpT* gene (1-16). 2% agarose gel at 50 volt for 40 minutes, then visualized under UV after staining with ethidium bromide.

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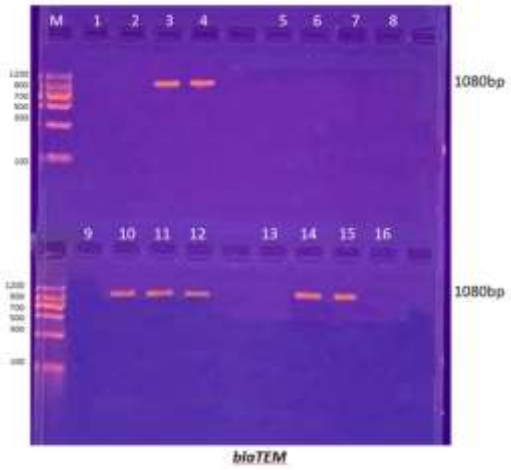

Fig 6: Gel Electrophoresis of PCR Product to *blaTEM* gene (1-16). 2% agarose gel at 50 volt for 40 minutes, then visualized under UV after staining with ethidium bromide.

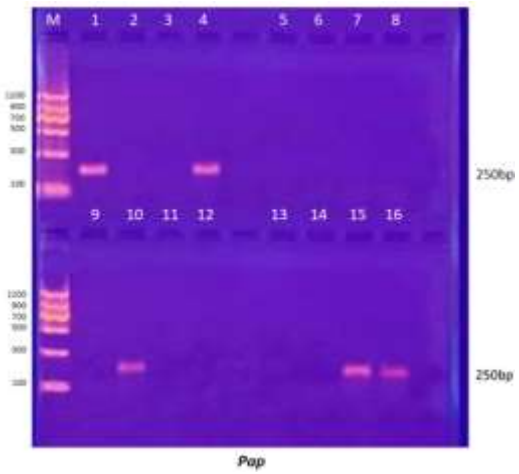

Fig 7: Gel Electrophoresis of PCR Product to *Pap* gene (1-16). 2% agarose gel at 50 volt for 40 minutes, then visualized under UV after staining with ethidium bromide.

In our study, the majority of bacterial isolates were obtained from urine samples (66.66%), followed by blood (12.82%), stool (12.25%), and wound swabs (10.25%). This finding aligns with previous reports that identified urinary tract infections (UTIs) as the most frequent bacterial infections, particularly caused by Escherichia coli [17]. The predominance of urinary isolates may be linked to the anatomical and physiological characteristics of the urinary tract, which make it a common target for bacterial colonization and infection [18]. Comparable results were reported by Getie et al[19] and Gupta et al[20], where urine represented the highest proportion of positive bacterial cultures among diverse clinical specimens .The antimicrobial susceptibility profile revealed a high sensitivity of isolates to meropenem (89.74%) and chloramphenicol (87.18%), whereas almost complete resistance was observed to amoxicillin-clavulanate (97.44%) and cefotaxime (82.05%). Moderate resistance was also detected against fluoroquinolones such as ciprofloxacin (74.36%), levofloxacin (56.41%), and norfloxacin (46.15%). These results indicate that carbapenems remain the most effective therapeutic option for treating severe infections caused by multidrug-resistant (MDR) Escherichia coli. Similar findings were documented by Kamruzzaman et al[21]. and Mishra et al. [22], who reported high efficacy of meropenem The production of extended-spectrum β-lactamases (ESBLs) was detected in 79.49% of bacterial isolates, while 20.51% were non-producers. This high prevalence indicates a substantial dissemination of β-lactamase–producing strains among clinical isolates, suggesting significant selective pressure caused by the frequent use of β-lactam antibiotics in healthcare settings. Comparable results were reported by Al-Hasani et al[23], who found an ESBL prevalence of 75% among E.coli isolates . Similarly, Mohammed et al[24]observed an 97% prevalence of ESBL producers in clinical isolates from various specimen types, In this study, (90%) were motile when tested on semi-solid medium, indicating the universal presence of functional flagella. Motility is an important virulence factor that enhances bacterial colonization, tissue invasion, and infection establishment, especially in urinary and wound infections. Similar findings were reported by Baral et al[25], where over 87% of E. coli isolates exhibited motility. The molecular detection showed that the OmpT was detected in all urine and wound samples, with lower prevalence in stool and blood isolates and blaTEM gene was detected in 50% of urine and blood isolates, and 75% of wound isolates, while stool isolates were negative. The Pap gene was present in 50% of urine isolates, 25% of wound isolates, and 50% of blood isolates, but absent in stool. These results suggest that urine and wound isolates carry a higher load of virulence and antibiotic-resistance genes, which may enhance their ability to colonize, adhere, and cause

infections.[26]These findings are Similar with Oh et al[27] showing that OmpT is commonly found in urine and Similar Mohammadzadeh et al [28] showing that blaTEM higher prevalence in urine and wound while present Helmy et al[29] Pap higher prevalence higher prevalence of these genes in urine and wound isolates highlights their role in pathogenesis and correlates with the observed antibiotic resistance patterns.

Conclusion

The study demonstrated significant variability in the distribution of virulence and resistance determinants among Escherichia coli clinical isolates. A high prevalence of extended-spectrum β-lactamase (ESBL) production was observed, emphasizing the clinical importance of monitoring β-lactamase-mediated resistance. Molecular detection revealed differential gene distribution, with ompT being the most prevalent across isolates, followed by blaTEM and Pap genes, particularly in urine and wound samples. These findings highlight the urgent need for continuous surveillance and prudent antibiotic use to limit the spread of multidrug-resistant E. coli strains.

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Author's Contributions

Noor N. Hassan sample collection, isolation, performed laboratory experiments, analyzed the data, and drafted the manuscript. Laheeb R. Hamad contributed to the study design, supervised the research work, and critically revised the manuscript for important intellectual content. Both authors read and approved the final version of the manuscript.

Ethics

The Ministry of Higher Education and Scientific Research, University of Fallujah, College of Applied Sciences, Department of Pathological Analysis for postgraduate studies Scientific Study Ethics Committee, Number AS-EC/0003 . Dated 2025/12/28 The Institutional Review Board approved experimental investigations involving humans or animals before the research began and samples were collected. In order to protect patient confidentiality and identity, no pictures of patients, healthy people, or parts of them were used.

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