



## RESEARCH ARTICLE

## Unveiling ESBL-Producing *E. coli*: Genetic Markers, Antibiotic Resistance, and Virulence Factors in UTI Patients Exhibiting MDR, XDR, and PDR Phenotypic bacterial isolates

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ARTICLE INFO	ABSTRACT
Received: May 29, 2024	<p>Background : Urinary tract infections (UTIs) are a worldwide issue that affect community and hospitalized individuals resulting in a decrease of the patient's life quality. Gram-negative bacilli are the most common etiological agents responsible for urinary tract infections. The prevalence of antibiotic resistance in Gram-negative bacilli is increasing at a rapid pace globally, which is constraining the available choices for UTI treatment. Materials and methods: This is a case-control study manipulated at City in Holy Karbala, Iraq, the study beginning from October 2023 to the end March 2024. Urine samples from patients suspected to have UTI were collected, and patients with confirmed UTI by laboratory investigations and yielded culture growth were enrolled. Antibiotic sensitivity testing and PCR testing of the EAE, Cnf, bla CTX-M and bla OXA-48 genes were done. Results: The study showed the distribution of extended- spectrum beta-lactamase (ESBL) producing <i>Escherichia coli</i> isolates based on gender, residency, and diabetes status. ESBL <i>E. coli</i> was more prevalent in females than males, more common in isolates from urban areas compared to rural areas, and isolated at a higher percentage in patients with diabetes than those without diabetes. For <i>E. coli</i> isolates, the highest percentage (43.6%) was observed in the (31-41 )years age group, compared to the other age groups. Conclusion: <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> were the most commonly isolated uropathogens in this study, and the majority were highly resistant to most of the antimicrobial agents tested. Resistance genes bla CTX-M, EAE , CNF and bla OXA-48 are very common in uropathogens, These results highlight the need for targeted surveillance and control measures, particularly in high-risk populations, to address the spread of ESBL-producing <i>E. coli</i> infections.</p>
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### INTRODUCTION

Urinary tract infection (UTI) a common health-care problem worldwide, urinary tract infection (UTI), represents a disease of significant impact on every country's economy.( Heidar *et al.*,2019). affecting 150 million people each year worldwide.( Paul *et al.* ,2024). Urinary tract infections (UTIs) are the most common outpatient infections, with a lifetime incidence of 50–60% in adult women (Medina and Castillo, 2019). as well as in males the incidence of UTIs was ranged in the ages under 50 years (Tan and Chlebicki ,2016) .

Normally urine is sterile and free of bacteria, viruses and fungi but does contain fluids, salts and waste products (Brunze, 2021). An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply (Komala and Kumar, 2013).

The bacteria which are responsible for causing UTIs such as *E.coli*, *Citrobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Providencia* spp., and *Morganella* spp, have more aggressive virulence factors which enhance their host cell attachment, colonization as well as invasion abilities (Olier *et al.*, 2017). These bacteria avoid evasion of the immune system of host by the help of certain virulence factors which may be comprised of various cellular components such as pili, capsule, lipopolysaccharides, various other cell surface structures and antimicrobial resistance (Johnson and Johnson, 2018).

The Enterobacteriaceae family includes many Gram-negative bacteria that live as normal flora inside the intestines of humans and animals (Oliveira *et al.*, 2017 ; Riedel *et al.*, 2019. Extended spectrum beta-lactamases (ESBL) are enzymes produced by members of the Enterobacteriaceae which can hydrolyze the beta-lactam antibiotics like mpenicillins and cephalosporins and thereby confer antibiotic resistance on strains producing them. bacterial isolates producing ESBLs have spread to different parts of the world. The ESBLs are encoded by several different genetic elements borne on the chromosome and plasmids (Dr. Uyanga *et al.*, 2020).

*CNF* is a virulence factor produced by some pathogenic *E. coli* strains that can induce morphological changes and cell death in host cells. The *CNF* gene encodes the *CNF* protein and is often associated with uropathogenic *E. coli* that cause urinary tract infections. ( Dersch *et al.* 2021) *OXA-type*  $\beta$ -lactamases are a class of enzymes that confer resistance to oxacillin and other  $\beta$ -lactam antibiotics in *E. coli* and other Gram-negative bacteria. The genes encoding these enzymes, such as *OXA-1*, *OXA-48*, and *OXA-181*, can be carried on plasmids or chromosomes in *E. coli*. The overexpression of *OXA-type*  $\beta$ -lactamases is a common mechanism of extended-spectrum  $\beta$ -lactam resistance in multi-drug resistant and extensively drug-resistant *E. coli* isolates (Ahmed *te al.*, 2-23) the aim of study was to Identify the genetic markers, antibiotic resistance profiles, and virulence factors associated with ESBL-producing *E. coli* isolates from UTI patients.

## METHODS

### Study group:

This case- control study was done at patients in hospital in Karbala province,. All patients collected were registered in UTI center in hospital from October (2023) to end of march (2024). Patients: 55 patients randomly recruited from the patients hospital in Karbala aged ranged between (20-63) years, who are diagnosed to have UTI based on clinical and laboratory findings(lymphocyte, neutrophils, eosinophils) by the clinicians; the patients data collection were include residence area, Diabetes mellitus and gender .

Controls: Healthy controls, 55 patients .With matched age and sex to the patients' group

**Inclusion criteria:** : All patients with urinary tract infection were diagnosed on the basis of clinical symptoms and other investigations

**Exclusion criteria:** The patients who have autoimmune diseases, cardiovascular disease, sepsis cancer, congenital urinary tract anomalies, Acute Kidney Injury, and others UTI patients with Bacterial growth than ESBL Enterobacteriaceae will be excluded. ESBL Gram positive bacteria.

**methods:** Patient data: Demographic and Clinical data will be collected using a specific detailed questionnaire . After that Sample collection from midstream urine specimens were collected from suspected patients in a sterile wide-mouth container and then transferred to the laboratory within two hours of collection from each participant and then centrifuged to get two phases sediment and supernatant , 50  $\mu$ l of sediment used for bacterial culture while the supernatant examined for dipstick test.

**Culture technique:** Well-mixed urine specimens (50 µl) were seeded on MacConkey agar, and EMB, chromogenic agar orientation plates, separately. They were incubated overnight at 37°C in bacteriological incubators under aerobic conditions. The identification of *E. coli* was done depending on morphological features, and the pink color of the colonies on MacConkey agar plates, confirmed by metallic green sheen on EMB agar, will give Primary identification of *E. coli*. A pure culture on chromogenic agar plates were made from each single group of *E. coli* colonies. The pure cultures were prepared for biochemical tests to confirm differentiation of *E. coli* from other lactose ferment Enterobacteriaceae (MacFadden, 2000).

### Molecular assays :

The study was investigate the presence and prevalence of specific genes associated with virulence and antibiotic resistance in Escherichia coli isolates, particularly from urinary tract infection (UTI) patients the study was used PCR assay to determine some virulence genes, which includes the key genes of interest are *CNF* (Cytotoxic Necrotizing Factor), *EAE* (*E. coli* Attaching and Effacing), *bla OXA-48* (*OXA-48* type β-lactamase), and *bla CTX-M* (Extended-Spectrum β-Lactamase). The primers used in our study were found in the table (1).

**Table 1: nucleotides sequences primers of some virulence genes**

Primer Name	Sequence	Reference
	5-3	
EAE -F EAE -R	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	Sousa, J. F. D. (2019)
BLA- CTM-F BLA- CTM-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	Poirel, L., etal.2001
CNF-F CNF-R	GCAGTCACCTGCCCTCCGGTA CATTCAAGATCCTGCCCTCATTATT	Nehmaa, S. A. (2023)
BLAOXA-48-F BLA-OXA-48-R	CCAAGCATTTTTACC CGCATCKACC GYTTGACCATACGCT GRCTGCG	Jalal Ahmed,etal.2023

All bacterial isolates were soaked in 500 µl of nuclease free water to DNA extraction procedure (Presto™ Mini gDNA Bacteria Kit/ Geneaid), PCR optimization were used for every primers as the following steps in table (2) :

Table(2): PCR optimization procedure:

Gene/ Names	Denaturation/ time	Denaturation/time	Anneling/time	Extension/ time	Final Extension/ min	Cycle Number
BLA-CTM	95 °C/ 5 min	95 °C / 30 sec	52 °C / 45 Sec	72 °C/ 1 min	72 °C/ 7 min	35

<b>BLA-OXA-48</b>	95 °C/ 5 min	95 °C / 30 sec	65 °C/ 45 Sec	72 °C/ 1 min	72 °C/ 7 min	35
<b>CNF</b>	95 °C/ 5 min	95 °C / 30 sec	66 °C/ Sec	72 °C/ 1 min	72 °C/ 7 min	35
<b>EAE</b>	95 °C/ 5 min	95 °C / 30 sec	55 °C/ Sec	72 °C/ 1 min	72 °C/ 7 min	35

The PCR product were run by used gel electrophoresis (1.5% agarose ) stained with ethidium bromide under 70 volts for 1 hour and visualization by UV transilluminator.

**Ethical approval:** The study protocol will be sent to the relevant ethical committee in the health directorate. Also, verbal approval will be taken from each participant before taking the sample. During samples collection, health measures and safety will be taken.

### RESULT:

#### Estimation the prevalence of ESBL bacterial isolate according to gender, residency and diabetes disease.

Figure (1) displays the distribution of ESBL *E. coli* according to gender, residency and diabetes. As the figure shows it was isolated at a higher percentage from females 53.8% than from males 46.2% and from urban areas 64.1% compared to rural areas 35.9%, and it was isolated at a higher percentage from patients with diabetes 69.2% compared with patients without diabetes:

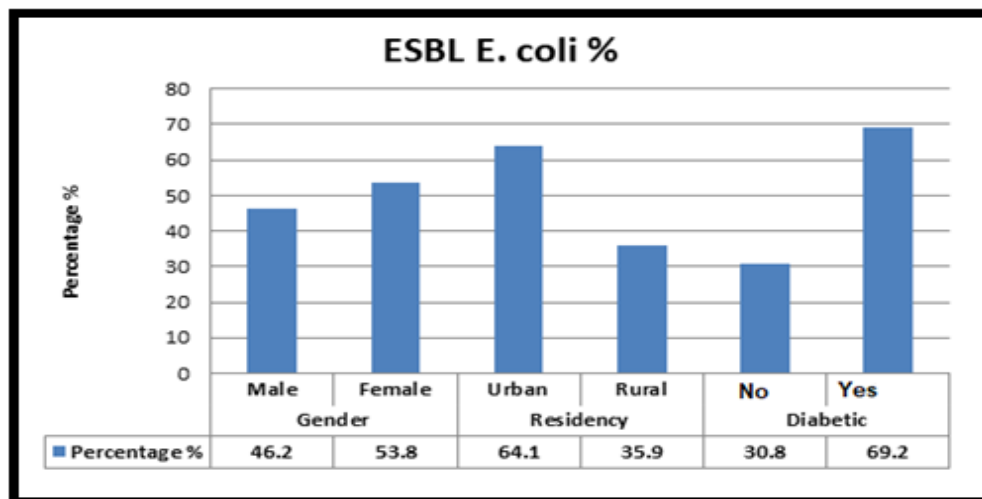


Figure (1): Estimation of the frequencies of UPEC bacterial isolate according to gender, residency and diabetes disease

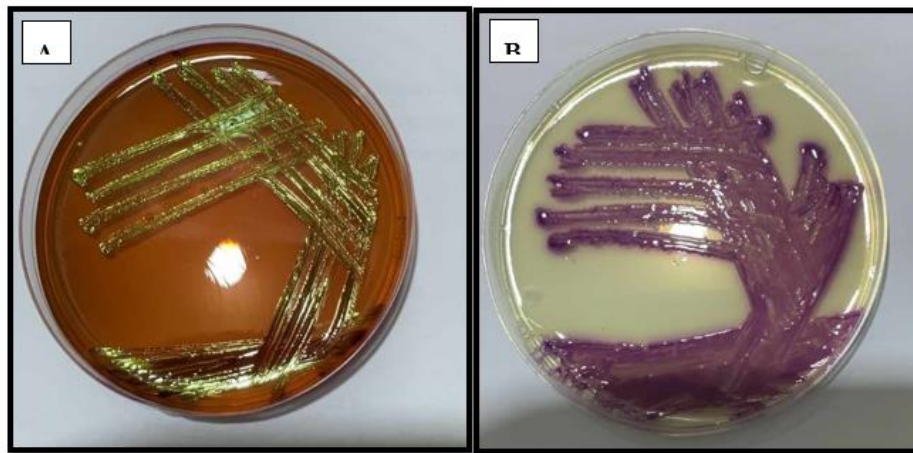
#### Distribution of uropathogenic bacterial isolate according to age groups

The current study also included the distribution of bacterial isolates according to the ages of the patients from whom these isolates were isolated. The patients were divided according to their ages into four age groups (20-30 y, 31-41 y, 42-52 y, 53-63 y) as illustrated in Table (3). In regard to *E.*

*coli*, the highest percentage 43.6% of them was isolated from (31-41 y) age group compared to other groups.

**Table (1): Distribution of bacterial Uropathogenic isolates according to age groups**

Types of isolates		Age group				Total
		20-30 y	31-41 y	42-52 y	53-63 y	
<i>Escherichia coli</i>	Count	13	17	4	5	39
	%	33.3%	43.6%	10.3%	12.8%	100.0%



**Figure (2) :Isolation of ESBL producing *E. coli* on A. EMB agar B.Chromogenic agar**

#### Phenotypic and genotypic characterization of ESBL UPEC isolates

A various levels of susceptibility were detected in UPEC isolates towards (11) antibiotics belonged to different classes by using VITEK system. The results revealed that antibiotic susceptibility test were affected at High rates of sensitivity were seen with cefepime (78.8%), Gentamicin (78.6%), Amikacin (76.2%), piper\Tazo and Nitrofurantoin (73.5%) and Amox/Clavu (72.2%); while High rates of resistance were seen with Ciprofloxacin (79.5%), Norfloxacin (76.9%), Ceftazidime (73.8%), and Ampicillin (70.9%); in addition, 100% of *E. coli* isolates have intermediate sensitivity to Amox/Clavu, cefotaxime, Gentamicin and Norfloxacin as well as 70% intermediate sensitivity to Ceftazidime, as showed in Table (3-9) and Figure (3.9). In the present study, PCR assay was done to detect the presence of specific *Bla-CTM*, *Bla OXA-48*, *CNF*, and *EAE* genes as virulence factor genes in UPEC (*E. coli*) isolates. The study revealed that the frequency of *Bla-CTM* in UPEC isolates were detected in all cases 39 (100%), *Bla OXA-48* and *CNF* were detected in 3 (7.7%), *EAE* were detected in 8 (20.5%), as detected and explained by Figure (3).

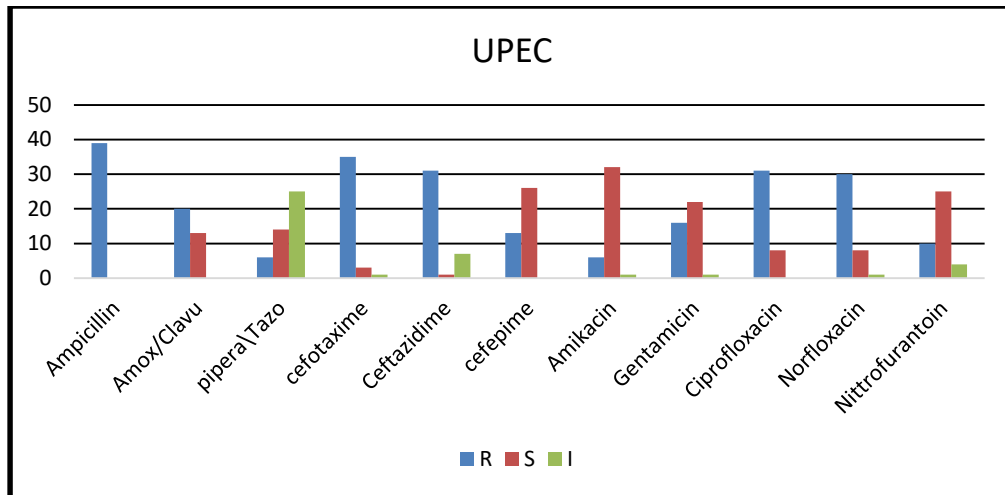


Figure (3): Type of antibiotic resistance patterns for UPEC of study isolates

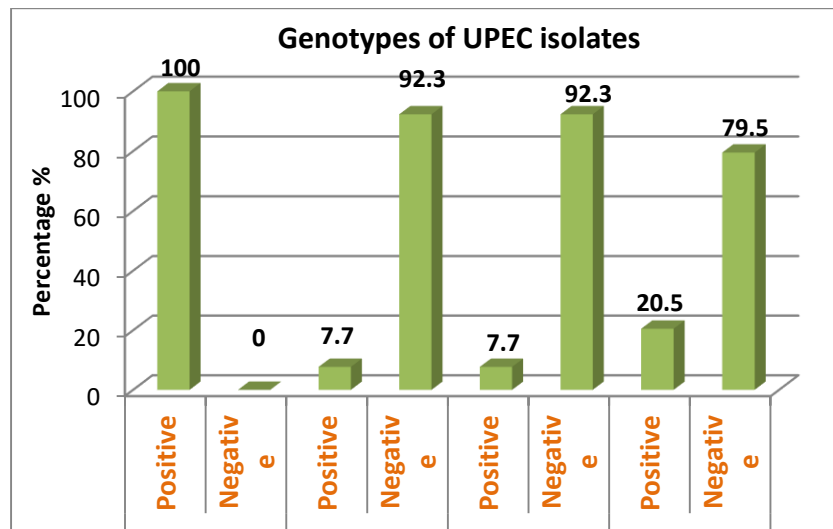


Figure (4): Virulence gene expression pattern in *E. coli* isolates



Figure (5): Identification of BLA- OXA 48 gene: the PCR product at band size 438bp. (Lane M:100 bp DNA marker, Lane 1-3 represent BLA- OXA



48 gene of ESBLs isolates visualized under ultraviolet light after ethidium bromide staining.

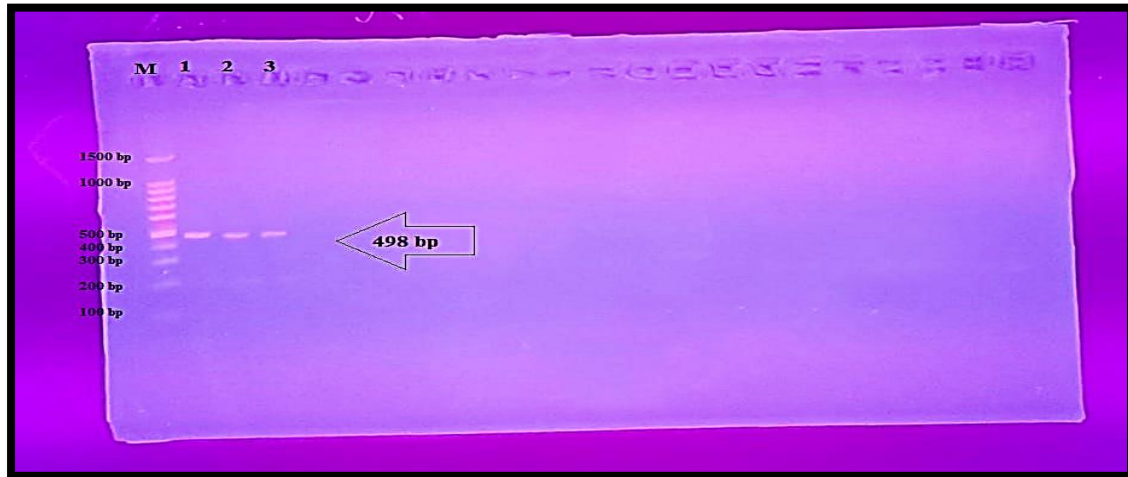


Figure (6): Identification of *CNF* gene the PCR product at band size 498 bp. (Lane M:100 bp DNA marker, Lane 1-3 represent *CNF* gene of ESBLs isolates visualized under ultraviolet light after ethidium bromide staining.

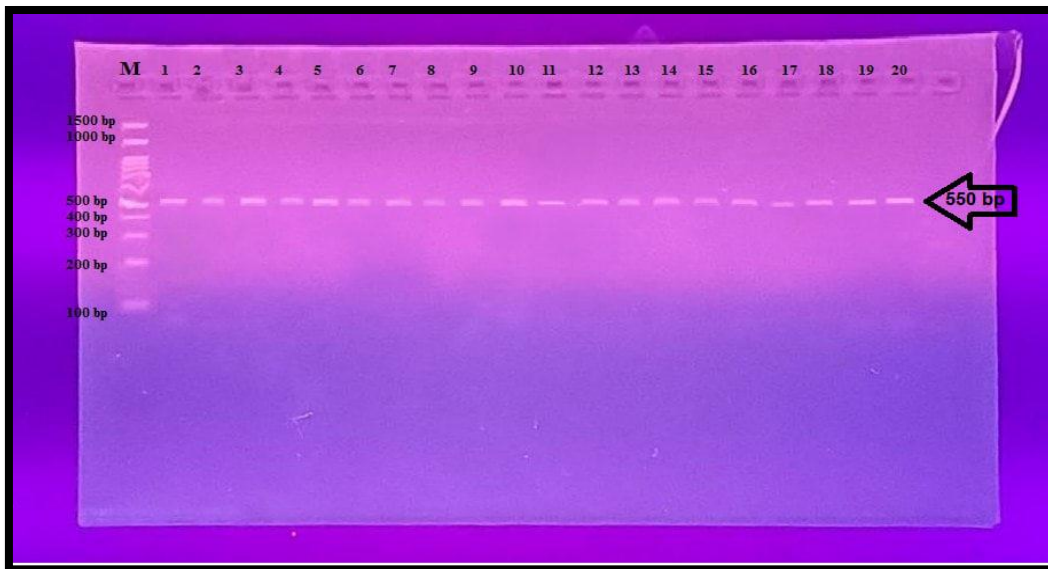
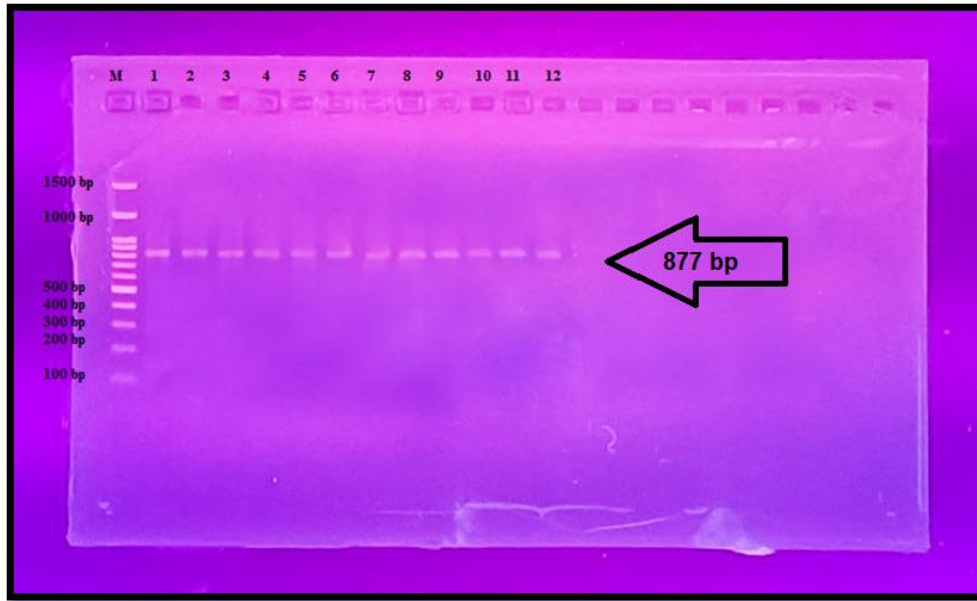


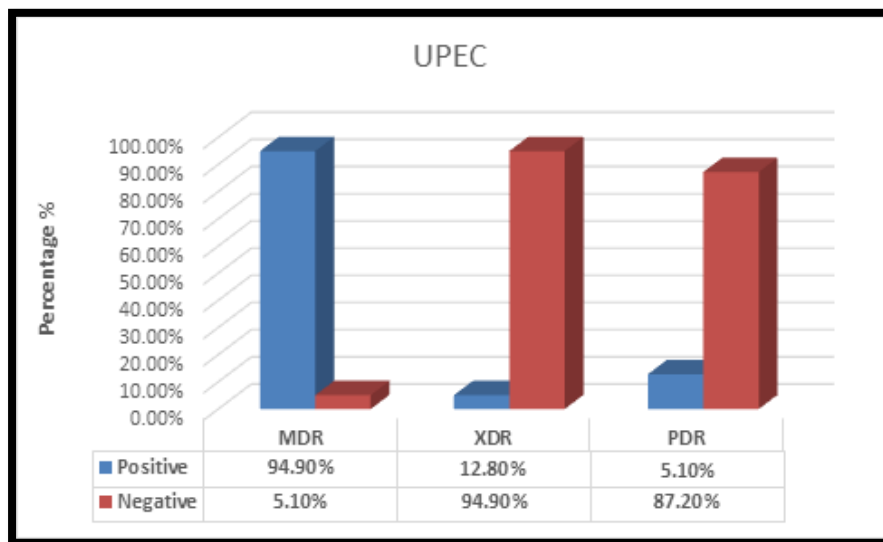
Figure (7): Identification of *BLA-CTM* gene the PCR product at band size 550 bp. (Lane M:100 bp DNA marker, Lane 1-3 represent *BLA-CTM* gene of ESBLs isolates visualized under ultraviolet light after ethidium bromide staining.



**Figure (8):**Identification of *EAE* gene the PCR product at band size 877 bp. (Lane M:100 bp DNA marker, Lane 1-3 represent *EAE* gene of ESBLs isolates visualized under ultraviolet light after ethidium bromide staining

**Evidence of MDR, XDR and PDR of ESBL of study isolate**

As shown in Figure (3.10), among UPEC *E.coli* isolates there are 37 (94.9%), 5 (12.8%), and 2 (5.1%) were MDR, XDR, and PDR respectively.



**Figure (9)**antibiotic resistance types in study isolated



## DISCUSSION

The pathogen was found to have a higher isolation rate in females (53.8%) compared to males (46.2%). The higher prevalence of UTIs caused by the pathogen in females compared to males can be attributed to anatomical and physiological differences between the two genders. Females have a shorter urethra compared to males, which means that bacteria have a shorter distance to travel to reach the bladder and cause an infection. Additionally, the urethra in females is closer in proximity to the anus, which can increase the risk of bacterial contamination from the gastrointestinal tract (Czajkowski *et al.*, 2021). Additionally, the pathogen was more frequently isolated in urban areas (64.1%) than in rural areas (35.9%), urban areas typically have higher population densities, with more people living near each other. This increased population density can facilitate the spread of infectious diseases, including UTIs caused by the pathogen. The higher concentration of individuals in urban areas increases the likelihood of contact and transmission of the pathogen (Kubone *et al.*, 2020). The importance of high population density in urban settings in hastening the spread of infectious illnesses is still highlighted by recent studies. For example, (Rocklöv and Sjödin 2020) draw attention to the ways that highly crowded metropolitan environments might accelerate the spread of infectious illnesses because of the greater human interaction and movement that occurs there. According to a research by (Zhang *et al.*, 2021), because germs may spread more easily in crowded healthcare facilities, the high patient turnover in urban hospitals raises the risk of nosocomial infections, including UTIs. Even while cities have generally superior infrastructure, there are still large differences in cleanliness and hygiene, especially in impoverished metropolitan areas (Ezeh *et al.* (2021) claim that a high population density in informal settlements and poor sanitation facilities aid in the development of infectious illnesses, such as UTIs. Antibiotic-resistant infections are becoming more common in urban areas due to the overuse and abuse of antibiotics, which makes treating UTIs more difficult. According to (Holmes *et al.* 2020), the increased incidence of antibiotic usage in metropolitan settings puts inhabitants there at danger of coming into contact with microorganisms that are resistant to antibiotics. The fast spread of diseases is facilitated by the high levels of migration and mobility found in metropolitan environments. Infectious illnesses spread more easily among urban dwellers and migrants who move in and out of cities on a regular basis. (Wu *et al.*, 2020) talk on how population movements brought about by urbanization are vital to the dynamics of infectious disease transmission. All things considered, new research confirms that a number of urban-specific characteristics, including high population density, healthcare use, differences in sanitation, antibiotic resistance, and high mobility, greatly contribute to the increased transmission of UTIs in urban environments. For urban populations to experience successful disease prevention and control, several challenges must be addressed.

On the other hand, the UTI pathogens were isolated at a higher percentage (69.2%) from patients with diabetes compared to patients without diabetes. This suggests that individuals with diabetes have a higher likelihood of being affected by UTIs caused by this particular pathogen and this is attributed to Diabetes can weaken the immune system and impair the body's ability to fight off infections, making individuals with diabetes more susceptible to UTIs and other infectious diseases. Additionally, elevated blood sugar levels in diabetes can create an environment that is favorable for bacterial growth, further more increasing the risk of UTIs (Ramrakhia *et al.*, 2020). In addition, diabetic cytopathy, a disorder that causes bladder dysfunction due to diabetic autonomic neuropathy, exists. These elements raise the risk of contracting infections. (Medicina, 2024). If the study results compared with other studies such as a comprehensive study and meta-analysis in Ethiopia revealed that the prevalence of UTIs in diabetes individuals was around 13.8%. This high incidence is caused by decreased immunological response, poor blood circulation, and bladder dysfunction (PLOS ONE, 2024). Similarly, a research in Saudi Arabia found a UTI incidence of 25.3% among diabetic patients, with significant risk variables including female sex, hypertension, and a higher BMI (Healthcare, 2024).

The present study observed a higher prevalence of urinary tract infections (UTIs) caused by uropathogenic bacteria in the age group of 42-52 years, compared to other age groups. This age group, which commonly includes people in their peri-menopausal and early post-menopausal years, has unique physiological and behavioral characteristics that contribute to an increased susceptibility to UTIs. This finding suggests that individuals within this age range are more susceptible to UTIs caused by these particular bacteria, Hormonal fluctuations that occur during perimenopause and menopause in women can affect the urinary tract, making it more susceptible to infections. These Changes in hormone levels can impact the protective mechanisms of the urinary tract, increasing the risk of UTIs (Aslam *et al.*, 2020). (Raz and Stamm, 2018) found that post-menopausal women have a higher incidence of UTIs due to estrogen insufficiency, which affects the urogenital flora and mucosal defenses. Also The immune system's ability to battle pathogens weakens with age, making middle-aged adults more vulnerable to infections, including UTIs. (Hilt *et al.*, 2020) found that older persons are more likely to get UTIs due to immunosenescence and other age-related physiological changes. as well as Age-related changes in the urinary tract, such as decreased muscle tone and thinning of the urethral lining, can impact the ability of the urinary tract to effectively flush out bacteria. This can increase the risk of bacterial colonization and subsequent UTI development (McCloskey *et al.*, 2024). Despite a decline in sexual behavior with age, sexual activity is still a substantial risk factor for urinary tract infections (UTIs) in middle-aged individuals. This is supported by a recent study conducted by (Ricoy-Cano *et al.*, 2024) which showed that people in their 42–52 age range who may still engage in sexual activity continue to have this risk factor, just like younger persons. This confirms earlier research on the link between sexual activity and the incidence of UTIs.

When ESBL-producing bacteria grow on chromogenic agar, the enzymes produced by these bacteria will cleave the chromogenic substrates. This enzymatic activity results in a color change, typically pink or reddish. The pink color is a visual indicator that the bacteria on the plate are producing ESBL enzymes (Djim, et al 2023)

Lactose fermentation, which results in acid production and a pH drop, is what gives *E. Coli* colonies on Eosin Methylene Blue (EMB) agar its metallic shine. This is because the dyes eosin and methylene blue precipitate. A crucial component of EMB agar's selective and differential growth inhibition of Gram-positive bacteria while promoting the growth of Gram-negative bacteria is this response, which is specific to powerful lactose fermenters such as *E. coli*.

Uropathogens growing resistance is limiting the efficacy of f prescription drugs in treating urinary tract infections. The treatment of UTIs is significantly hampered by antibiotic resistance. because it limits the possibilities for therapy, raises the chance of treatment failure, and has negative economic effects, such as higher healthcare costs, expenditures brought on by a rise in hospital admissions and drug use The results revealed that different antibiotics within the family of drugs showed varying rates of sensitivity and resistance in the tested *E. coli* isolates. Cefepime, Gentamicin, Amikacin, piperacillin/tazobactam, and Nitrofurantoin exhibited high rates of sensitivity, while Ciprofloxacin, Norfloxacin, Ceftazidime, and Ampicillin showed high rates of resistance, Cefepime belongs to the cephalosporin class of antibiotics, resistance to cephalosporins can occur through  $\beta$ -lactamase production, which enzymatically inactivates the drug.

Bacteria can produce different types of  $\beta$ -lactamases, such as extended-spectrum  $\beta$ -lactamases (ESBLs), which can confer resistance to cefepime (Mushtaq *et al.*, 2021). Gentamycin and Amikacin antibiotics belong to the aminoglycoside class, the resistance to aminoglycosides can arise through enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs) that chemically modify the drug, reducing its efficacy (Aishwarya *et al.*, 2020). Several researchers reported that a high percentage of isolated uropathogens emphasized antibiotics resistance issue and this beside current study, sending out a clear warning sign to optimize therapy in accordance with the resistance profile

and carry out public interventions to contain the problem's spread. (Subramaniyan,etal.,2021; Joya,etal;2022; Herbawi,etal.,2024)

According to a research done in Sudan, 19.5% of patients had bacteriuria. The most common bacterial isolate, *Escherichia coli*, had a significant degree of resistance to several medicines, underscoring the difficulty of treating UTIs in areas where antibiotic resistance exists (Mohammedkheir *et al.* , 2024). The evolution of antimicrobial resistance emphasizes the need for new antibiotic development or other ways in order to successfully tackle these illnesses, hence further complicating treatment options.

The Resistance of ESBLs to fluoroquinolones can occur through mutations in the target enzyme DNA gyrase or topoisomerase IV, reducing the drug's binding affinity and efficacy, and Ceftazidime is another cephalosporin antibiotic. Resistance to ceftazidime can be mediated by  $\beta$ -lactamases, including ESBLs or AmpC  $\beta$ -lactamases. In some cases, bacteria can also exhibit reduced permeability of the outer membrane, limiting the entry of the antibiotic (Wang *et al.*, 2020).

The *Bla-CTM* gene was detected in all cases of UPEC isolates, indicating a 100% prevalence. *Bla-CTM* is associated with the production of  $\beta$ -lactamases, which can confer resistance to  $\beta$ -lactam antibiotics like cephalosporins and penicillins, on the other hand The *Bla OXA-48* gene and CNF gene were detected in 3 isolates, indicating a frequency of 7.7%. *Bla OXA-48* is associated with resistance to carbapenem antibiotics, while CNF (cytotoxic necrotizing factor) is a virulence factor that can contribute to the pathogenicity of UPEC. The *EAE* gene was detected in 8 isolates, indicating a frequency of 20.5%. *EAE* (intimin-encoding gene) is associated with the ability of *E. coli* to adhere to and invade host cells, contributing to its virulence (Obodoechi *et al.*, 2020).

The results indicates a universal presence of the *Bla-CTM* gene across all bacterial isolates tested (100% in all species). This finding is consistent with several studies that have reported a high prevalence of *Bla-CTM* genes in various bacterial species, often linked to extended-spectrum beta-lactamase (ESBL) production, which confers resistance to a broad range of beta-lactam antibiotics . The ubiquitous presence of *Bla-CTM* in our isolates underscores the need for stringent antibiotic stewardship and the development of novel therapeutic strategies to combat these resistant strains, In contrast to *Bla-CTM*, the *Bla OXA-48* gene was found in only a small fraction of *Escherichia coli* isolates (7.7%) and was completely absent in other bacterial species tested. This lower prevalence aligns with other studies where *Bla OXA-48*, a carbapenemase gene, is less commonly found but poses a significant threat due to its role in carbapenem resistance, particularly in Enterobacteriaceae . The limited presence in our sample could be due to geographical variations or the specific clinical settings from which the isolates were obtained.

The *CNF* (cytotoxic necrotizing factor) gene was present in 7.7% of *Escherichia coli* isolates and universally present in *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. *CNF* genes are known for their role in virulence, contributing to pathogenicity by disrupting host cell signaling . The differential distribution observed suggests species-specific factors influencing the acquisition and retention of *CNF* genes, warranting further investigation into the genetic mechanisms and environmental factors at play. Finally, The *EAE* (*E. coli* attaching and effacing) gene, associated with the ability to form attaching and effacing lesions, was found in 20.5% of *Escherichia coli* isolates but showed varied presence across other species, being entirely absent in *Acinetobacter baumannii*. This gene is a key virulence factor in enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) . The relatively higher prevalence in *Escherichia coli* is consistent with its known role in intestinal infections, while its presence in non-Enterobacteriaceae species highlights the potential for horizontal gene transfer and the evolution of pathogenic traits.

The figure reveals that MDR is the most prevalent resistance phenotype, with a positive rate of 94.90%, indicating that a significant proportion of the UPEC isolates are resistant to multiple types of antibiotics. This high level of MDR is concerning and suggests the need for comprehensive antibiotic stewardship programs and the development of alternative treatment strategies to manage infections caused by these resistant strains (Ibrahim *et al.*, 2012). In contrast, the XDR phenotype, which represents an even higher level of resistance, has a relatively lower positive rate of 12.8%, although the presence of XDR isolates is still a significant concern, as these strains are resistant to most, if not all, available antibiotics, leaving limited treatment options for clinicians. So the study disagreement with (Al-Hasani *et al.*, 2023) who was found the percentage of XDR 1.17% of multiple drug resistance from *E. coli* isolated from Iraqi clinical isolates among patients in Baghdad city. The PDR phenotype, which denotes resistance to all tested antibiotics, has a positive rate of 5.81%. The emergence of PDR strains is particularly alarming, as they leave healthcare providers with no effective antibiotic options to treat the associated infections, potentially leading to increased morbidity and mortality this study was agreement with (Datok *et al.*, 2021) who was found PDR was 4.6% in *Escherichia Coli* isolated from barbecued beef (Suya) sold in a Nigerian City.

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