Molecular characterization of blaTEM and blaCTX-M ESBLs genes producing Escherichia coli isolates from urinary tract infections (UTIs) in Al-Basrah province, Iraq

Ahmed. J. Mohammed[1], Saad S. Mahdi Al-Amara[2], Murtakab Y. Al- Hejjaj[3]

[1]*Department of Biology, College of Science, University of Basrah, Iraq.*

[2]*Department of Pathological analyses, College of Science, University of Basrah, Iraq.*

[3]*Department of Microbiology, College of Veterinary medicine, University of Basrah, Iraq.*

KEYWORDS
E.coli , ESBLs, blaTEM and blaCTX-M genes

ABSTRACT
Urinary tract infections (UTIs) are primarily caused by Gram-negative Enterobacteriaceae bacteria, accounting for 80–90% of uropathogenic Escherichia coli infections. Multidrug-resistant (MDR) bacteria, such as extended-spectrum β-lactamases (ESBLS), pose a significant threat to global healthcare systems. The current study relied on 200 urine samples collected from patients suffering urinary tract infections (UTIs) in Al Sadr Teaching Hospital in Al-Basrah Province,Iraq from the 5th January to 22nd February. A result of cultivated 200 samples collected from urinary tract infection patients showed 71 (35.5%) positive samples on MacConkey agar, eosin methylene blue (EMB), and HiChromeTM E.coli agar, and bacterial growth was distributed to 47 (66.2%) Escherichia coli and 24 (33.8%) of Gram-negative species. The diagnostic gene 16S rDNA by PCR method showed that all 47 E. coli isolates had a molecular weight of around 585 bp at 100%. Furthermore, positive results were shown in 44 (93.6%) of the E.coli isolates in the current study that produced ESBLs by using the double-disc approximation method (DAM). While 3 (6.4%) isolates revealed negative results for ESBLs, whereas the13(27.7%) of the E.coli isolates gave positive results for ESBL. While 34(72.3%) E.coli isolates showed negative results for produced ESBLs by using the double-disc synergy test (DDST), there were significant differences (P<0.01) between positive and negative isolates in both methods used to detect ESBL-producing isolates. Extended-spectrum β-lactamses (ESBLs) genes were detected by using PCR to amplify the blaTEM and blaCTX-M genes in E.coli isolates. The amplified genes' bands were characterised approximately at (800 bp) for blaTEM and (754 bp) for blaCTX-M , the products were compared to the standard molecular DNA ladder at (2000 bp). According to the results, 47(100%) E.coli isolates gave positive results for the blaTEM gene. While blaCTX-M gene was shown, only 23(48.9%) E.coli isolates had positive results for the blaCTX-M gene.

1. Introduction

In primary care, urinary tract infections (UTIs) are among the most frequent bacterial illnesses reported [1]. Urinary tract infections (UTIs) can occur in a variety of ways and places, ranging from acute, symptomatic infections to asymptomatic bacteriuria. UTIs are frequently brought on by bacteria that live on the skin or are a component of the gut flora [2]. Gram-negative bacteria within the Enterobacteriaceae family, which account for 80–90% of uropathogenic Escherichia coli infections, are thought to be the main cause of UTIs [3]. ESBLs the best type of medication for treating pathogenic bacteria is an antibiotic. When treating infections brought on by Gram-negative bacteria, doctors typically administer fluroquinolones, cephalosporins, β-lactams, and β-lactamases inhibitors either alone or in combination [4]. One major problem threatening the global healthcare system is multidrug-resistant (MDR). Usually, the widespread Enterobacteriaceae family is linked to the problem. Extended-spectrum β-lactamases (ESBLS), are the main antimicrobial resistance mechanism in this family of bacteria that renders β-lactam antibiotics ineffective [5].

Gram-negative bacteria have capable of produced enzymes hydrolyzing the bonds in β-lactam rings, which has allowed them to become resistant to one of the most successful drugs: β-lactams [6]. In 1983, extended-spectrum β-lactamases (ESBLs) were identified in Germany and demonstrated the ability to hydrolyze penicillin's, monobactams, and cephalosporins. Since then, the number of diseases caused on by bacteria that possess this resistance mechanism has increased [7,8]. Furthermore, E. coli has evolved resistance to several antibiotics, leading to a major cause of illness [9]. The public's health is seriously threatened by the rapidly spreading multidrug-resistant (MDR) E. coli strains, particularly those that produce extended-spectrum β-lactamase (ESBL) [10,11]. These

include the blaTEM, blaSHV, and blaCTX-M type ESBLs, which may hydrolyse antibiotics such as cephalosporins, monoclonal antibodies, and penicillins. blaCTX-M have emerged as the predominant ESBLs for E. coli strains globally throughout the previous 20 years[12,13].These drug-resistant genes can restrict available treatments, raise morbidities and deaths, lengthen hospital stays, and result in substantial financial consequences as a result of their production and dissemination [14].This study aimed to determine the frequency of ESBL-producing Gram-negative bacteria, their antimicrobial, and the molecular identification of ESBL genes blaTEM and blaCTX-M in E. coli isolate among UTIs patients , in Al-Basrah province ,Iraq.

2. Methodology

Collection of specimens:

From the 5th January to 22nd February, 200 urine samples were collected from patients suffering urinary tract infections (UTIs) in Al Sadr teaching hospital at Al-Basrah province ,Iraq.

-Isolation and identification

The samples were cultivated for 24h at 37^{O}C on nutrient broth , and after that cultured on MacConkey agar, Eosin Methylene Blue agar (EMB) according to [15,16], furthermore the Hi-chrome^{TM} *E.coli* agar to confirm identify *E. coli* isolates.

- DNA extraction

Genomic DNA extracted from isolates according to (Wizard® Genomic DNA Purification Kit, Geneaid , Taiwan) kit protocol.

- Detection of 16S rDNA

The isolate DNA extraction was amplified by PCR for *16S rDNA* amplification with a particular primer that was approximately (585 bp) in length [17]. Standard molecular DNA ladder (2000 bp) was used compare the PCR results.

-Detection of Extended Spectrum β-lactamase (ESBL)

-Double disk synergy test (DDST)

According to CLSI recommendation this test was performed. The Mueller-Hinton agar plates were used to cultivate the *E.coli* isolates to determine if they produced extended spectrum β-Lactamase (ESBLs) enzymes. 5ml bacterium inoculum was prepared and compared with 0.5 McFarland suspension. Synergy between two antibiotics of each third generation cephalosporin 30µg disk and Amoxicillin- Clavulanate (20µg/10µg) were placed center to center with distance (30 mm) on Muller Hinton agar. When one of these antibiotics disks showed inhibition zones towards Amoxicillin-Clavulanate disk, the isolate was considered positive for ESBL production [18]

-Double disk approximation method (DAM)

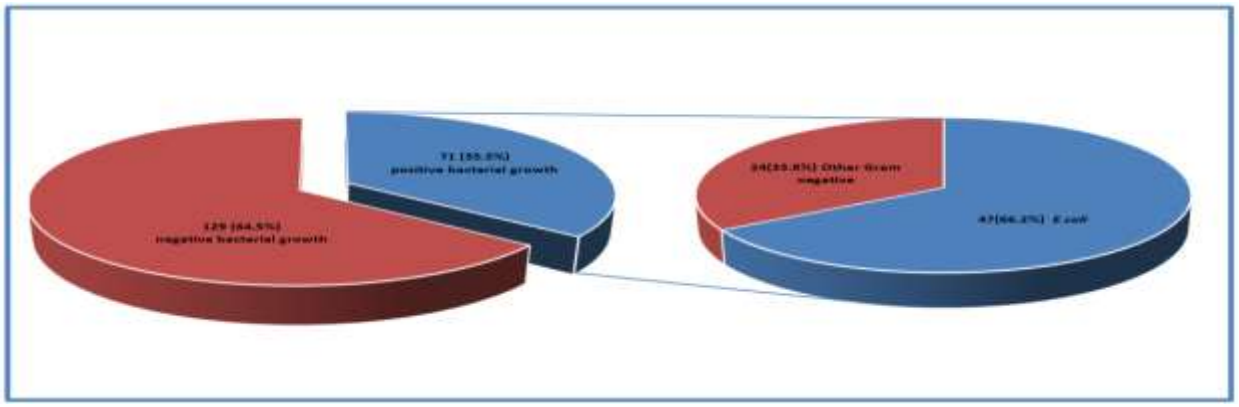
The Amoxicillin- Clavulanate (20µg/10µg) disk was placed on center of the agar plate, four antibiotics disks due to the third generation cephalosporin and monobactam such as aztreonam(30ug), ceftriaxone(30µg), ceftazidime (30µg) and cefotaxime (30µg) were used and placed around it at distance about (20mm center to center). Plates were incubated for 24h at 37c and results detect according to[18,19].

-Detection of ESBLs Genes

Two different primers that were utilized for amplified bla_{TEM} and bla_{CTX-M} genes by PCR with a particular primer that was approximately (800 bp) and (874 bp) in length respectively according to [20,21]. Standard molecular DNA ladder (2000 bp) was used compare the PCR results.

3. Results and discussion

In the current study, the results of cultivated 200 samples that collected between the 5th January to 22nd February, from patients suffering urinary tract infections were gave 71 (35.5%) samples positive; and on MacConkey agar, eosin methylene blue (EMB) and HiChrome™ *E. coli* agar. The bacterial growth was distributed to 47 (66.2%) *Escherichia coli* and 24 (33.8%) other Gram-negative species figure (1). While 129 (64.5%) samples were gave negative results for bacterial growth. The result in current study was showed significant differences P<0.01 between isolates of *E.coli* compared to other Gram negative isolates by using ANOVA test (one way). Furthermore, the results of molecular diagnostics employing the PCR technique, which relies on the diagnostic gene *16S rDNA*, revealed that all n= 47 *E.coli* isolates (100%) gave a positive results for detected of *16SrDNA* in a molecular weight approximately in 585bp figure (2).



Figure(1): the distribution of positive and negative samples according to bacterial growth

Figure (2): Agarose electrophoresis patterns show PCR amplified products of 16S rDNA. Lane1:(2000 bp DNA ladder), Lane:(no. 1-6) 16S rDNA band of E.coli isolates using 1% agarose gel, 70V, 1h.

and frequency of bacterial isolates in current study.

Furthermore, positive results were showed the 44(93.6%) out of the n=47 *E.coli* isolates in the current study produced extended-spectrum β-lactamases (ESBLs), by using the double-disc approximation method (DAM).While 3(6.4%) isolates revealed negative results for ESBLs. Whereas the results in current study were showed the13 (27.7%) out of n=47 *E.coli* isolates gave positive for ESBL .While the 34(72.3%) *E.coli* isolates were showed negative results for produced ESBLs by using the double-disc synergy test (DDST).The results of the statistical analysis was showed significant differences P<0.01 between positive and negative isolates in both methods that used to detect ESBL producing isolates.

PCR was used to amplify the bla_{TEM} and bla_{CTX-M} genes in *E.coli* isolates. The amplified genes' bands were characterized approximately at (800 bp) for bla_{TEM} and (754 bp) for bla_{CTX-M}, the products was compared to the stander molecular DNA ladder at (2000 bp). According to the results, 47(100%) *E.coli* isolates had gave positive results for the bla_{TEM} gene as in figure (3). While bla_{CTX-M} gene was

shown that only 23(48.9%) *E.coli* isolates had positive results for bla_{CTX-M} gene as in figure (4).

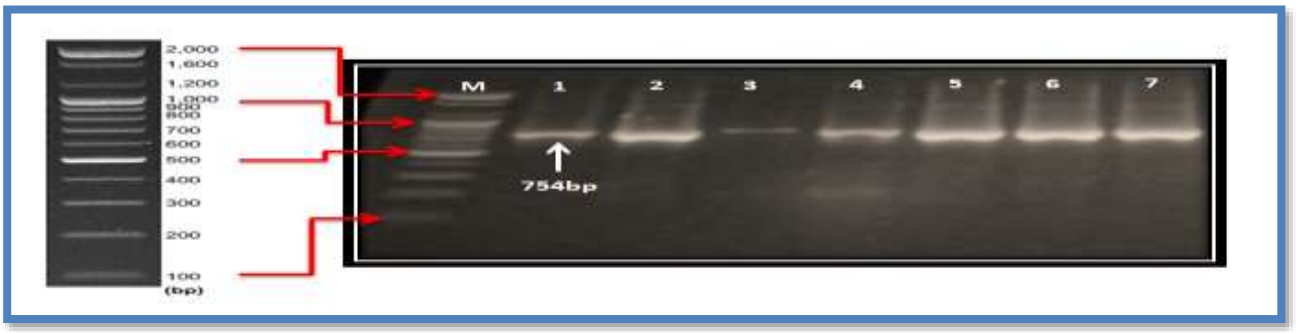


Figure 3: Agarose electrophoresis patterns of *TEM* gene PCR amplified products. Lane1:(2000 bp DNA ladder), Lane:(no. 2-8) *TEM* gene bands of *E.coli* isolates. using 2% agarose gel, 70V, 1h.

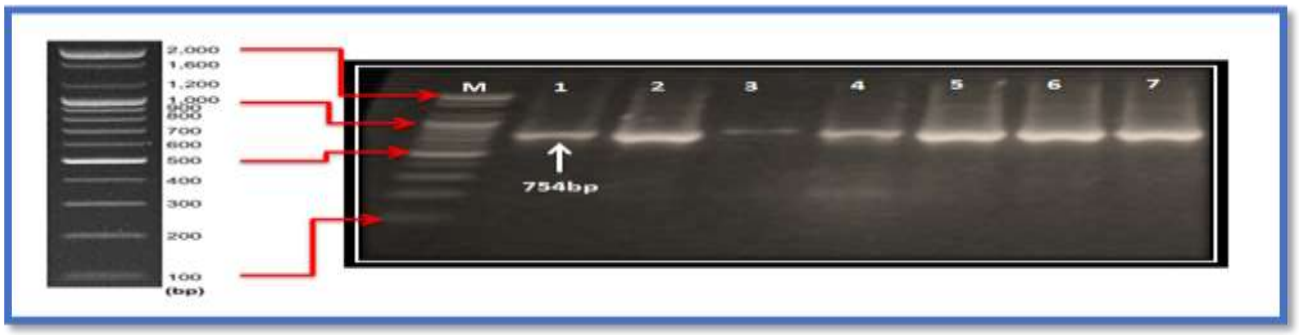


Figure 4: Agarose electrophoresis patterns of *CTX-M* gene PCR amplified products. Lane1:(2000 bp DNA ladder), Lane:(no. 2-8) *CTX-M* gene bands of *E.coli* isolates using 2% agarose gel, 70V, 1h.

Discussion:

E. coli spreads in the urinary tract due to its natural habitat in the digestive tract, its anus opening near the urinary tract opening, and its virulence characteristics, which allow it to stick to the surface, withstand urine-induced drift, and prevent urinary tract infections. Current study's results revealed the prevalence of *E. coli* bacteria in urinary tract infections (UTIs), and they were in line with the findings of a study performed in Baghdad, Iraq, by[22], which found that 38% of cases of UTIs were caused by *E. coli*, making it the most frequently isolated bacterial pathogen. According to study of [23]in Duhok, Iraq, 170 (80.6%) of the isolates were Gram-negative bacteria and 41 (19.4%) were Gram-positive bacteria. The most frequent source of infection was *E. coli*. The study of [24] in Al-Basrah, Iraq was reported the *E. coli* common costive agent of urinary tract infections (UTIs). Whereas the study by [25] in Zakho, Iraq was reported the *K. pneumonia* common causative agent of urinary tract infections (UTIs).

Current study's results was showed the production of ESBLs are in accordance with research carried out in study of [26,27,28,29&30]. When the double-disc approximation techniques (DAM) were utilized in studies by [31]in India and [32]in Nepal, the results differed from the results of the current study. On the other hand, the findings of this study, which used the double-disc synergy test (DDST) to identify the producer of ESBLs, are in accordance with research done in [33,34&35].β-lactamase, which helps break down the β-lactam ring in certain medications, is one major virulence feature that helps *E. coli* grow more virulent and resistant to antibiotics[36,37&38]. Extended-spectrum ß-lactamases (ESBLs) have been identified in recent years as a major contributor to antibiotic resistance in Enterobacteriaceae, which includes resistance to ß-lactamases. ESBL enzymes are resistant to penicillin's, monobactams, cephalosporins, and other antibiotic families [35].

By utilizing the PCR method in current study, all 47 (100%) of the *E. coli* isolates in this

investigation produced positive results for the identification of the bla_{TEM} gene. Statistical analysis revealed no significant differences P>0.05 between the isolates that tested positive or negative for the bla_{TEM} gene. In studies [30, 36], the bla_{TEM} gene was found to be 100% and 75%, respectively. In study [45], it was stated that every *E. coli* isolate under investigation had the bla_{TEM} gene 100%. However, according to a research by [39,40,41,42, and 43], bla_{TEM} gene was present in 6.8%, 48.7%, 28.6%, 40%, and 36.4% of *E.coli* isolates. The bla_{TEM} gene was primarily responsible for resistance to cephalosporins, including cephalothin and cephaloridine, as well as penicillin. As a result of changing the amino acids in the bla_{TEM} activation region, more than over 130 distinct variants of *TEM* β-lactamases. Whereas results in the current study showed that out of n=47 *E.coli* isolates 23(48.9%) isolates gave positive results for bla_{CTX-M} gene. While the 24(51.1%) isolates were showed negative results for bla_{CTX-M} by using the PCR technique, and results showed no significant differences P>0.05 between positive and negative isolates of bla_{CTX-M} gene.

The study by [36,37&39] were found that all *E.coli* isolates have bla_{CTX-M} gene in ratio (100%) , on other hand the study of [32]reported *E.coli* isolates have bla_{CTX-M} gene in ratio (66.1%) and study of [29] found that bla_{CTX-M} genes in *E.coli* isolated from urine samples were (37.2%).The remarkable ability of the bla_{CTX-M} gene products to hydrolyze cefotaxime, ceftazidime, and aztreonam might perhaps account for the elevated occurrence of the bla_{CTX-M} variation. According to earlier research, bla_{CTX-M} is the most common β-lactamase found in UPEC isolates [43,44]. The ST131-H30 strains of $bla_{CTX-M\ -15}$ *E. coli* that first surfaced in 1990 and the subsequent global spread of community-acquired ESBL-producing *E. coli* of the bla_{CTX-M} type are primarily responsible for the sudden rise in the number of ESBL-producing Enterobacteriaceae. Most infections caused by ESBL-producing bacteria were caused by bla_{TEM}-ESBL and bla_{SHV}-ESBL. Following bla_{CTX-M} emergence, the coexistence of these ESBL genes increased resistance even further and increased the spread of Enterobacteriaceae carrying mobile genetic elements connected to multiple virulence factors and antibiotic resistance genes that cause invasive infections in public and healthcare settings [45].There are several commonalities in the emergence of bla_{TEM}- and bla_{SHV}-type ESBLs. Since antibiotics are widely used and often taken at random, the high incidence of third-generation cephalosporin resistance isolates in the current investigation may be related to this. In order to give effective antibiotic therapy, it is essential to remain up to date on the predominant resistance pattern of any area, since the spread of MDR and ESBL-producing *E. coli* isolates limits treatment options and increases hospital consumption [46].

4. Conclusion and future scope

The blaTEM gene in E. coli isolates is linked to medication resistance, posing a significant threat to global healthcare systems. Gram-negative bacteria, particularly those producing extended-spectrum β-lactamase (ESBL), pose a threat to horizontal gene transfer, posing a serious threat to public health.

Reference

[1] Pouwels, K. B., Dolk, F. C. K., Smith, D. R., Robotham, J. V., & Smieszek, T. (2018). Actual versus 'ideal'antibiotic prescribing for common conditions in English primary care. *Journal of Antimicrobial Chemotherapy*, 1(73):19-26

[2] Gilbert, N. M., O'Brien, V. P., & Lewis, A. L. (2017). Transient microbiota exposures activate dormant Escherichia coli infection in the bladder and drive severe outcomes of recurrent disease. *PLoS pathogens*, 13(3):1-19.

[3] Jaafer Mezal, T., AH Ajeel, N., & J Hasony, H. (2011). Antimicrobial resistance of uropathogens in Basrah. *The Medical Journal of Basrah University*, 29(1), 13-18.

[4] Alhamedy, A. J., & Shani, W. S. (2020). Determination of Cathelicidin in UTI patients of Basrah province. *Annals of Tropical Medicine and Public Health*, 23(2): 182-189.

[5] Hardany, M. J., Al-Abdullah, A. A., Al-Amara, S. M., & Makki, H. M. (2019). Detection ESBL enzymes in gram-negative bacteria. *Journal of Basrah Researches ((Sciences))*, 45(2):53-59.

[6] Bush, K.; Bradford, P.A. Epidemiology of β-Lactamase-Producing Pathogens. Clin. Microbiol. Rev. 2020, 33(2)1-37.

[7] Sarowska, J., Choroszy-Krol, I., Jama-Kmiecik, A., Mączyńska, B., Cholewa, S., & Frej-Madrzak, M. (2022). Occurrence and characteristics of carbapenem-resistant *Klebsiella pneumoniae* strains isolated from hospitalized patients in Poland—A single centre study. *Pathogens*, 11(8): 859.

[8] Nicolas-Chanoine ,M.h.,Bertrand,X., Madec,J.Y.(2014) *Escherichia coli* ST131,an intriguing clonal group.Clin. Microbiol.Rev, 27(3):543-74.

[9] Trecarichi,E.M.,Cauda,R.,Tumbarello,M.,(2012).Detecting risk and predicting patient mortality in patients with extended-spectrum β-lactamase-producing Enterobacteriaceae bloodstream infections. Future microbiol. 7(10):1173-89.

[10] Kanwar,N.,Scott,H.M.,Norby,B.,Loneragan,G.H.,Vinasco,J.,McGowan,M.*et al*., (2013).Effects of ceftiofur and chlortetracycline treatment strategies on antimicrobial susceptibility and on tet(A), tet(B0, and bla CMY-2resistance genes among E.coli isolated from feces of feedlot cattle .PLoS One,8(11):1-13.

[11] Mathers,A.J.,Peirano,G.,Pitout,J.D.(2015).The role of epidemic resistance plasmids and international high-risk clonse in the spread of multidrug-resistance Enterobacteriaceae .Clin.Microbiol. Rev. 28(3): 565–591.

[12] D'Andrea,M.M., Arena,F.,Pallecchi,L.,Rossolini,M.G.(2013).CTX-M-type β-lactamase:a successful story of antibiotic resistance.Int. J. Med. MICROBIOL. 303(6-7):305-17.

[13] Burrows LL (2024) It's uncomplicated: prevention of urinary tract infections in an era of increasing antibiotic resistance. PLoS Pathog 20(2):1-9.

[14] Ali T, Ahmed S, Aslam M (2023) Artificial intelligence for antimicrobial resistance prediction: challenges and opportunities towards practical implementation. Antibiotics (basel) 12(3):523.

[15] Holt, J.J., Krieg, N.R., Sneath, B.H.A., Staley ,J.T. and Williams ,S.T. (1994). Bergey's manual determinative bacteriology. 9th Ed. Williams and Wilken, Baltimore, 175-248.

[16] Harly, J.P. & Prescott , L.M.(2002). Laboratory Exercises in microbiology. 5th Edition,The McGraw -Hill Companies ,Inc.,New York.

[17] Salama, S. (2017). Differentiation between vaccinal and field strains ofE. coli using phenotype and genotype characterization. Journal of Immunology and Clinical Microbiology, 2(3), 48-53.

[18] Clinical and Laboratory Standards Institute (CLSI). (2020). Performance Standards for Laboratory Antimicrobial Testing, 30th ed. Wayne, Pennsylvania, USA.

[19] Coelho, N. T. A., da Silva, R. S., Delmondes, G. M., Lima, W. G., de Matos Jensen, C. E., & de Paiva, M. C. (2021). Occurrence of extended-spectrum betalactamase (ESBL) and carbapenemases among ampicillin-resistant enterobacteriales recovered from a municipal raw sewage in Minas Gerais, Brazil. *Revista Colombiana de Ciencias Químico-Farmacéuticas*. 50(3):708-725.

[20] Perez, F.; Hujer,A.M.; Hujer, K. M.; Decker, B.K. and Rather, P.N. (2007). Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother., 51(10):3471-3484.

[21] Ramachandran, A., Shanthi, M., & Sekar, U. (2017). Detection of blaCTX-M extended spectrum beta-lactamase producing Salmonella enterica serotype Typhi in a tertiary care centre. Journal of Clinical and Diagnostic Research: JCDR, 11(9): 21–24.

[22] Saad R., N. A. A., Hussein, R. H., & Al-Shakir, N. M. (2022). Antibiogram Pattern of Uropathogenic Escherichia Coli in Baghdad Province, Iraq. Journal of Techniques, *4*(Special Issue), 2708 (4):134-138.

[23] Naqid, I. A., Balatay, A. A., Hussein, N. R., Ahmed, H. A., Saeed, K. A., & Abdi, S. A. (2020). Bacterial strains and antimicrobial susceptibility patterns in male urinary tract infections in Duhok province, Iraq. *Middle East Journal of Rehabilitation and Health Studies*, *7*(3):1-6.

[24] Jalil, M. B., & Al Atbee, M. Y. N. (2022). The prevalence of multiple drug resistance Escherichia coli and Klebsiella pneumoniae isolated from patients with urinary tract infections. Journal of Clinical Laboratory Analysis, 36(9):1-7.

[25] Polse, R. F., Qarani, S. M., Assafi, M. S., Sabaly, N., & Ali, F. (2020). Incidence and Antibiotic Sensitivity of Klebsiella pneumonia isolated from urinary tract infection patients in Zakho emergency hospital/Iraq. *Journal of Education and*

Science, 29(3), 257-268.

[26] Sadeghi, M., Ebrahim-Saraie, H. S., & Mojtahedi, A. (2022). Prevalence of ESBL and AmpC genes in *E. coli* isolates from urinary tract infections in the north of Iran. *New Microbes and New Infections*, *45*, 100947.

[27] Ai, I., Rafaque, Z., Ahmed, S., Malik, S., & Dasti, J. I. (2016). Prevalence of multi-drug resistant uropathogenic Escherichia coli in Potohar region of Pakistan. *Asian Pacific Journal of Tropical Biomedicine*, 6(1), 60-66.

[28] Hashemizadeh, Z., Kalantar-Neyestanaki, D., & Mansouri, S. (2018). Clonal relationships, antimicrobial susceptibilities, and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from urinary tract infections and fecal samples in Southeast Iran. *Revista da Sociedade Brasileira de Medicina Tropical*, 51(1):44-51.

[29] Naziri, Z., Derakhshandeh, A., Soltani Borchaloee, A., Poormaleknia, M., & Azimzadeh, N. (2020). Treatment failure in urinary tract infections: a warning witness for virulent multi-drug resistant ESBL-producing Escherichia coli. *Infection and drug resistance*. 13:1839-1850.

[30] Hassuna, N. A., Khairalla, A. S., Farahat, E. M., Hammad, A. M., and Abdel-Fattah, M. (2020). Molecular characterization of Extended-spectrum β lactamaseproducing *E. coli* recovered from community-acquired urinary tract infections in Upper Egypt. Scientific Reports, 10(1), 1-8.

[31] Suresh, M.; Nithya, N.; Jayasree, P. and Kumar, M. P. (2016). Detection and Prevalence of Efflux Pump-Mediated Drug Resistance in Clinical Isolates of Multidrug-Resistant Gram-Negative Bacteria from North Kerala,India. Asian J Pharm Clin Res. 19(3): 324-327.

[32] Pandit, R., Awal, B., Shrestha, S. S., Joshi, G., Rijal, B. P., & Parajuli, N. P. (2020). Extended-spectrum β-lactamase (ESBL) genotypes among multidrug-resistant uropathogenic *Escherichia coli* clinical isolates from a teaching hospital of Nepal. *Interdisciplinary perspectives on infectious diseases*.

[33] Sadeghi, M., Mojtahedi, A., Nikokar, I., & Roushan, Z. A. (2023). The emergence of plasmid-encoded oxacillinase and carbapenemase among uropathogenic Escherichia coli (UPEC) isolated from hospitalized patients in the North of Iran. *Heliyon*, *9*(4).

[34] Shamami, A. M., Anvari, M., Pourmoshtagh, H., Shafighi, S. T., & Ebrahim-Saraie, H. S. (2023). Serogroup and Pathogenicity Island Marker Distributions Among Uropathogenic Escherichia coli Isolates in Rasht, Iran. *Jundishapur Journal of Microbiology*, *16*(1):1-8.

[35] Ibrahim, M. E., Algak, T. B., Abbas, M., & Elamin, B. K. (2021). Emergence of bla TEM, bla CTX-M, bla SHV and bla OXA genes in multidrug-resistant Enterobacteriaceae and *Acinetobacter baumannii* in Saudi Arabia. *Experimental and Therapeutic Medicine*, *22*(6), 1-11.

[36] Al–Ezee, A. S. M., Al–Taai, H. R. R., & Sultan, A. A. (2019). Occurrence oh blaSHV, blaCTX-M, blaTEM and Integrons genes in *Escherichia coli* isolates from urinary tract infection. athesis. *College of Education for pure science/Diyala University*.

[37] Albadery, A. A, Al-Amara, S. S. , Al-Abdullah, A, A (2023) Phenotyping and Genotyping Evaluation of E. coli Produces Carbapenemase Isolated from Cancer Patients in Al-Basrah, Iraq. Arch Razi Inst. 2023 Jun; 78(3): 823–829.

[38] Hardany ,M. J. , Al-Abdullah, A. A. , Al-Amara, S. S. , Makki, H. M.(2020). molecular investigation of Gram negative bacteria extended spectrum β-lactamase in haemodialysis patients in basrah province, Iraq. Plant Archives Vol. 20 : 1573-1576.

[39] Daniel M. , Gunturu R. and Andrew C. W. (2017); Molecular characterization of multidrug-resistant Klebsiella pneumonia and Escherichia coli harbouring extended spectrum beta-lactamases and carbapenemases genes at a tertiary hospital, Kenya. Microbiologia Medica . 32(70-76):132-137.

[40] Trupti Bajpai,M. Pandey, M. Varma, and G. S. Bhatambare1.(2017). Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital.AVICENNA JOURNAL OF MEDICINE. 2017 Jan-Mar; 7(1): 12-16.

[41] Al-Kudhairy, M. K., and Alshammari, M. M. M. (2019). Extended spectrum βlactamase-producing *Escherichia coli* isolated from pregnant women with asymptomatic UTI in Iraq. EurAsian Journal of BioSciences, 13(2), 1881–1889.

[42] Mahmoud, A. T., Salim, M. T., Ibrahem, R. A., Gabr, A., & Halby, H. M. (2020). Multiple drug resistance patterns in various phylogenetic groups of hospital-acquired uropathogenic *E. coli* isolated from cancer patients. *Antibiotics*, *9*(3), 108.

[43] Nabti, L. Z., Sahli, F., Radji, N., Mezaghcha, W., Semara, L., Aberkane, S.,…& Godreuil, S. (2019). High prevalence of multidrug-resistant *Escherichia coli* in urine samples from inpatients and outpatients at a tertiary care hospital in Setif, Algeria. *Microbial Drug Resistance*, *25*(3), 386-393.

[44] Halaji, M., Shahidi, S., Atapour, A., Ataei, B., Feizi, A., & Havaei, S. A. (2020). Characterization of extended-spectrum β-lactamase-producing uropathogenic *Escherichia coli* among Iranian kidney transplant patients. *Infection and drug resistance*, *13*, 1429.

[45] Logan, L. K., Rispens, J. R., Medernach, R. L., Domitrovic, T. N., Hujer, A. M., Marshall, S. H.,…& Bonomo, R. A. (2021). A Multi-Centered Study of the Clinical and Molecular Epidemiology of TEM-and SHV-type Extended-Spectrum Beta-Lactamase producing Enterobacterales Infections in Children. *The Pediatric infectious disease journal*, *40*(1): 39.

[46] Jena, J., Sahoo, R. K., Debata, N. K., & Subudhi, E. (2017). Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β-lactamase-producing Escherichia coli strains isolated from urinary tract infections in adults. *3 Biotech*, *7*(4), 1-7.