ISSN Print: 2664-6188
ISSN Online: 2664-6196
Impact Factor: RJIF 5.35
IJCBB 2023; 5(2): 26-30
www.biochemistryjournal.net
Received: 19-06-2023
Accepted: 27-07-2023

Aseel Ali Albadery
Department of Biology, College of Science, University of Basrah, Iraq

Amani Abd-Al-Ridha Al-Abdullah
Department of Pathological Analyses, College of Science, University of Basrah, Iraq

Saad Shakir Mahdi Al-Amara
Department of Pathological Analyses, University of Basrah, Iraq

Corresponding Author:
Aseel Ali Albadery
Department of Biology, College of Science, University of Basrah, Iraq

Investigation of β-lactamase-producing *Escherichia coli* from cancer patients

Aseel Ali Albadery, Amani Abd-Al-Ridha Al-Abdullah and Saad Shakir Mahdi Al-Amara

DOI: https://doi.org/10.33545/26646188.2023.v5.i2a.46

Abstract
Background: *E. coli* is nosocomial bacteria which leading infections of the urinary tract in the patients with cancer. The emergence of β-lactam-resistant strains is a major source of concern in UTI treatment. resistant to β-lactam *E. coli* is becoming more of an issue everywhere, including in Iraq. The study estimate β -lactam-resistant *E. coli* prevalence in Basrah province/ Iraq.
Methods: The isolated uropathogens were detected by Vitek®2 technique, and the *E. coli* sensitivity to antibiotics was assessed. Additionally, *E. coli* were examined using the DAM and DDST techniques, and polymerase chain reaction (PCR) was used to find the main β -Lactamase genes blaTEM, blaOXA, blaCTX-M, and blaSHV.
Results: The urine samples were collected from patients with cancer (120) from Basrah center of the Oncology Al-Sader Teaching Hospital, who were suspected with UTIs. Biochemical tests used to identify bacterial growth have revealed a variety of bacterial species, with *E. coli* isolates accounting for the majority of cases (53.6%) and the other types (19.34%), respectively. The findings of this investigation revealed that among (n=22) *E. coli* isolates, the12 (54.55%) of the isolates produced extended spectrum-lactamases (ESBLs) with positive results. While employing the double-disc approximation method, the results for the 10 (45.45%) isolates showed no evidence of ESBL production. Polymerase chain reaction and double-disc synergy were used to detect the *TEM*, *CTX-M*, *SHV*, and *OXA* genes encoding ESBL. The percentage of genes found in the isolates under study were (100%), (31.8%), (100%), and (100%), respectively
Conclusions: There is a spread of multiple types of ESBLs *E. coli* from UTIs cancer patients in Basra hospitals, and the isolated *E. coli* have a high ability to produce ESBLs against the third generation of cephalosporins and monobactam antibiotics.

Keywords: ESBLs, *E. coli*, UTIs, cancer

Introduction
Immunodeficiency in cancer patients is a common problem, and it puts them at a higher risk of contracting various infections due to the treatment or the itself cure [1, 2, 3]. The urinary tract infection is associated with the cancer [4]. UTS are commonly caused by bacteria, which arise when there are more than 100,000 colony-forming units in 1 mL of urine, but they can also be caused by viruses and fungi, such as candida. All the types of the bacteria can cause infection, which is followed by clinical signs [5-6].
UTI is caused common by Enterobacteriaceae, with uropathogenic Escherichia coli (UPEC) at 80-90% of all infections [7, 8]. Followed by *K. pneumonia*, *Staph. spp*., Candida spp., *E. faecalis*, *Streptococcus*, *Proteus mirabilis*, and *P. aeruginosa*, respectively [9]. UPEC vary from commensal strains in that they have gained virulence and resistance determinants through plasmids, bacteriophages, pathogenicity islands, or transposons' DNA horizontal transfer, allowing them to colonize the urinary tract effectively and cause a wide range of diseases [7]. One of the most serious dangers of the twenty-first century is third-generation resistant Enterobacteriaceae, which includes Enterobacteriaceae that produce ESBLs. According to WHO [10]. The presence of genes that produce β-lactamase enzymes in Gram-negative bacteria is mechanisms of antibiotic resistance [11]. Extended-spectrum β-lactamases (ESBLs) hydrolyze many β-lactam antibiotics, causing failure in the bacterial infections treatment [12-13].

Materials and Methods

Collection of specimens

The study was done (October 2021 to January 2022), by taking the urine samples (120) were obtained from cancer patients at the Oncology Al-Sader Teaching Hospital's Basrah center, with patients in age (18-84) years old and suspected of UTIs. The samples were given to 50 (41.7%) men and 70 (58.3%) women.

Isolation and identification

All the *E. coli* isolates were detected based on Forbes *et al.* [14] and Harley and Prescott [15]. the step (1) and (2) are involved the identification by Vitek.

Antibiotic sensitivity test: B-lactam resistance was diagnosed by Aztreonam (30µg) Amoxicillin- Clavulanate (30µg) Cefotaxime (30µg) Ceftazidime (30µg) and ceftriaxone (30µg) (Liofilchem com.) disc according to the method of (16-17) (the Vitek®2 techniques was used to detect the antibiotic susceptibility of isolates.

Molecular Detection

DNA Extraction

DNA was extracted from *E. coli* isolates by DNA kit (Geneaid). By using agarose, DNA bands were determined.

PCR for the Detection of β-Lactamase genes

Detection of β-Lactamase genes was done by primers using, it designed for bla_{TEM}, bla_{OXA}, $bla_{SHV,}$ $bla_{CTX\text{-}M,}$ and, The primers we are provided by (Macrogen, Korea) Table (1).

Table 1: The used primers for β-lactamase genes

Primers		DNA Sequences (5'-3')	Product size bp	Reference
bla TEM	F	CATTTCCGTGTCGCCCTTATTC	800	18
	R	CGTTCATCCATAGTTGCCTGAC		
blaSHV	F	AGCCGCTTGAGCAAATTAAAC	713	18
	R	ATCCCGCAGATAAATCACCAC		
blaCTX-M	F	CGCTGTTGTTAGGAAGTGTG	754	19
	R	GGCTGGGTGAAGTAAGTGAC		
blaOXA	F	ATATCTCTACTGTTGCATCTCC	619	20
	R	AAACCCTTCAAACCATCC		

PCR amplification

PCR was done in volume of 25l, 1 F and 1 R primer, DNA template (2), nuclease-free water (8.5), PCR master mix (12.5) (Promega/USA). PCR device (Bioneer/Korea) set up for DNA amplification, as the followed stages: the initial denaturation and denaturation are done at 94 °C, for five min and half min respectively, the annealing (at 60 °C, blaTEM; 58 °C, blaSHV; 56 °C, blaOXA;60 °C, blaCTX-M-1) for 35 sec, and the extension for half minute at 72 °C, the final extension for nine min at 72 °C (19-21-22)

Results

The urine sample (120) was taken from the patients with cancer at age (18-84), who have UTIs. The samples were distributed to 50 (41.7%) males and 70 (58.3%) females. The positive urine samples were 41/120 (34.2%), while the negative culture were 79 (65.8%). The bacterial Identification are done by the biochemical tests, *E. coli* was most frequent 22 (53.65%), as for the other types, it was 19 (46.34). The current study's findings revealed that (n=22) *E. coli* isolates The 12 (54.55%) isolates developed ESBLs, and the results were positive. While DAM and DDST method demonstrated negative results for ESBLs generated in the 10 (45.4%) isolates (Figure 1)

PCR technology was detecting the *TEM*, *SHV*, *CTX-M*, and *OXA* genes encoding ESBL The genes percentage of *E. coli* were (100%), (31.8%), (100%), and (100%), respectively. figure (2).

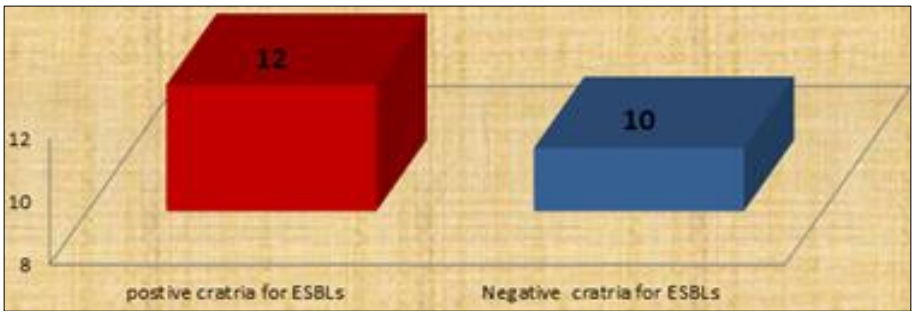
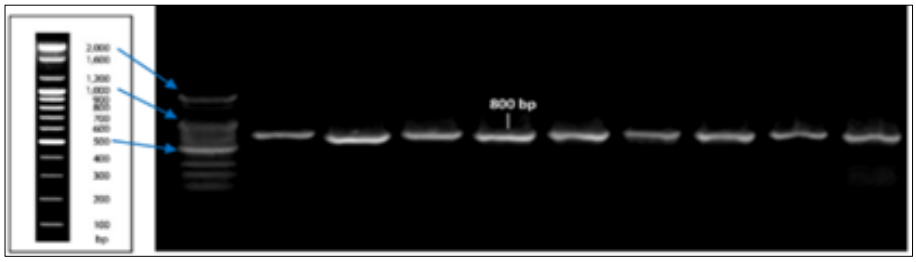
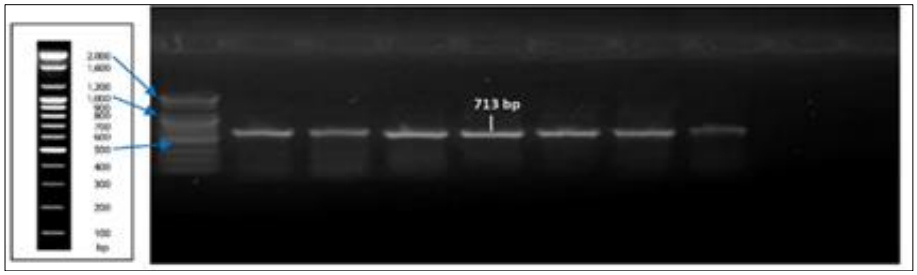


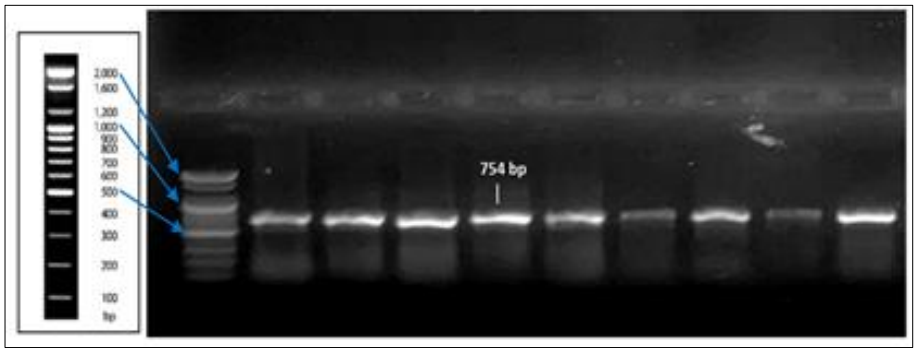
Fig 1: Using (DAM) and (DDST), *E. coli* produces both (+) and (-) for extended-spectrum β -lactamases (ESBLs)



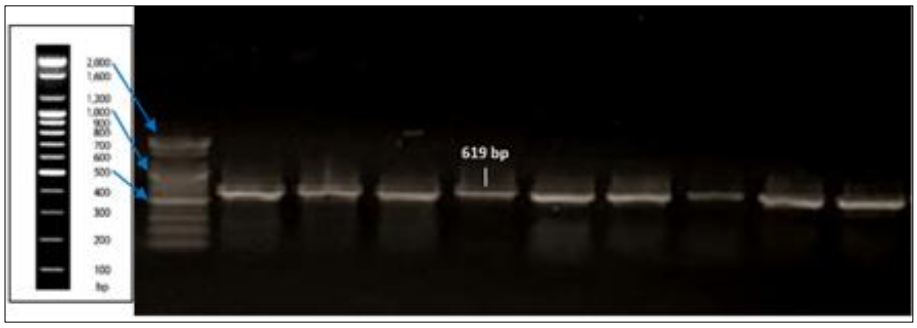
A



B



C



D

Fig 2: PCR for the Detection of β-Lactamase genes, shows PCR products of β-Lactamase genes. Lane L: ladder, A: *TEM* bands of *E. coli* Lane: (no. 1-9), B: *SHV* band of *E. coli*. Lane: (no. 1-7), C: *CTX-M* bands of *E. coli* Lane: (No. 1-9) D: *OXA* bands of *E. coli* Lane: (no. 1-9)

Discussion

Due to their immunosuppression, cancer patients are more likely to get life-threatening opportunistic infections. Due to excessive antibiotic use and lengthy hospital stays [23]. Because of their chronic immunosuppression, illnesses are associated with in the cancer [4]. Our results are included that *E. coli* was common bacteria in UTIs at (53.65%). which is consistent with the previous study. by Al-naimi, & Abbas [21]. The study reported the results as follows; *E. coli* 21 (31.82%) isolates the present study agreed with Shrestha *et al*. [23], *E. coli* was most rate bacteria (58%). Also agreed with Islam *et al*. [25], who observed *E. coli,* 53%. One reason for *E. coli* dominance over other bacterial species that cause UTIs is that it is part of the normal flora in the intestines, where it can enter the urinary tract and cause infection due to its possession of virulence factors such as biofilm, fimbria, adhesins, alpha-hemolysin, iron acquisition systems, and cytotoxic necrotizing factor as well as genes that rendered it resistant to antibiotics. Almost all β -lactam antibiotics [26].

β -lactamase is a key virulence factor that aids in the destruction of lactam ring in some antibiotics, increasing antibiotic resistance and virulence in *E. coli* [27-28]. The findings of the present investigation concur with those of a study conducted in Egypt by Hassuna *et al*. [36] who discovered that ESBL isolates rate was (59.7%). The present study came close to the study of Belete [30], discovered that 66.7% of their isolates in Ethiopia produced ESBLs. While Pandit *et al*. [31] found that ESBL production was at (40.3%), and the current study did not accord, in current study, the widespread use of antibiotics and the haphazard administration of antibiotics may be to blame for the high prevalence of third-generation cephalosporin resistant isolates. Because ESBL- *E. coli* increases hospital expenditures and decreased treatment options, it is vital to stay current on any locality's resistant pattern for offer appropriate antibiotic therapy [32]. *E. coli* get resistance genes and virulence genes horizontality. One of the most significant HGT agents, conjugative plasmids, may spread effectively and widely among many bacterial species. Plasmids with both virulence and antibiotic resistance genes are severe hazard to public health [33].

One of Middle Eastern nations categorized as developing is Iraq. The resistant genes are spread in the environment, animals and human. Many factors contribute to the spread of multidrug resistant bacteria, including: In drug stores, the sale of antibiotics is not limited, and customers are able to purchase them off the shelf. Due to inadequate sanitation, lax control measures, and inappropriate use of antibiotics in animals and humans, Iraq is experiencing significant and worrying levels of bacterial drug resistance. Iraq also experienced economic and military crises throughout the

past 20 years, which resulted in significant population shifts between Iraqi cities and its neighbors. These national issues contributed to a loss of control over sanitary measures and the prevention of infectious disease transmission [34-35].

Acknowledgments
The current work was supported by the University of Basrah, Science College, Biology department, Iraq. There are no competing interests.

References

1. Taur Y, Pamer EG. Microbiome mediation of infections in the cancer setting. Genome medicine. 2016;8(1):1-7.
2. Hadi DA. Study of Bacterial Infections and the Role of some Host Biomarkers in Early Detection among Febrile Pediatric Cancer Patients under Chemotherapy in Basrah Province- Iraq. University of Basrah; c2020.
3. Abdul-Ridha LA. isolation and identificaton of Bacterial DNA Integrated in the DNA of Cancer Patients. University of Basrah; c2017.
4. Tigabu A, Ferede W, Belay G, Gelaw B. Prevalence of asymptomatic bacteriuria and antibiotic susceptibility patterns of bacterial isolates among cancer patients and healthy blood donors at the University of Gondar Specialized Hospital. International Journal of Microbiology; c2020.
5. Marshall CW, Kurs-Lasky M, McElheny CL, Bridwell S, Liu H, Shaikh N. Performance of Conventional Urine Culture Compared to 16S rRNA Gene Amplicon Sequencing in Children with Suspected Urinary Tract Infection. Microbiology spectrum. 2021;9(3):e01861-21.
6. Xu R, Deebel N, Casals R, Dutta R, Mirzazadeh M. A new gold rush: A review of current and developing diagnostic tools for urinary tract infections. Diagnostics. 2021;11(3):479.
7. Kaur N, Agarwal A, Grover M, Singh S. Uropathogenic Escherichia.In(Ed.),Enterobacteria[Working Title]. Intech Open; c2022. http://doi.org/10.5772/intechopen.102525.
8. Alhamedy AJ, Shani WS. Determination of Cathelicidin in UTI patients of Basrah province. Annals of Tropical Medicine and Public Health. 2020;23:182-189.
9. De Nisco NJ, Neugent M, Mull J, Chen L, Kuprasertkul A, de Souza Santos M, *et al*. Direct detection of tissue-resident bacteria and chronic inflammation in the bladder wall of postmenopausal women with recurrent urinary tract infection. Journal of molecular biology. 2019;431(21):4368-4379.
10. World Health Organization. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis (No. WHO/EMP/IAU/2017.12). World Health Organization; c2017.
11. Kindye E. Extended spectrum beta lactamase producing bacteria among patients with ear infection at Felege Hiwot Referral Hospital, North West Ethiopia (Doctoral dissertation); c2022.
12. Ahmadi M, Ranjbar R, Behzadi P, Mohammadian T. Virulence factors, antibiotic resistance patterns, and molecular types of clinical isolates of Klebsiella Pneumoniae. Expert Review of Anti-infective Therapy. 2022;20(3):463-472.
13. Hardany MJ, Al-Abdullah AA, Al-Amara SM, Makki HM. Detection ESBL enzymes in gram-negative bacteria. Journal of Basrah Researches ((Sciences)). 2019;45(2):53-59.
14. Forbes BA, Sahm DF, Weissfeld AS, Brawn IA. Bailey and Scotts, Diagnostic Microbiology. 12th ed. Mosby. USA. Inc St. Louis; c2007.
15. Harly JP, Prescott LM. Laboratory Exercises in microbiology. 3th ed WCB, McGraw Hill; c2002.
16. Khan MKR, Thukral SS, Gaind R. Evaluation of a modified double-disc synergy test for detection of extended spectrum β-lactamases in AMPC β-lactamase-producing Proteus mirabilis. Indian journal of medical microbiology. 2008;26(1):58-61.
17. CLSI: Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 21 informational supplement. CLSI. Document M100-S21, Wayen, P A. USA; c2011.
18. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrobial agents and chemotherapy. 2007;51(10):3471-3484.
19. Ramachandran A, Shanthi M, Sekar U. 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, 2017, 11(9), DC21.
20. Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. Journal of clinical and diagnostic research: JCDR. 2013;7(10):2173.
21. Ogutu JO, Zhang Q, Huang Y, Yan H, Su L, Gao B, *et al*. Development of a multiplex PCR system and its application in detection of blaSHV, blaTEM, blaCTX-M-1, blaCTX-M-9 and blaOXA-1 group genes in clinical Klebsiella pneumoniae and Escherichia coli strains. The Journal of antibiotics. 2015;68(12):725-733.
22. Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. Distribution of bla CTX− M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrobial Resistance & Infection Control. 2019;8(1):1-10.
23. Shrestha G, Wei X, Hann K, Soe KT, Satyanarayana S, Siwakoti B, *et al*. Bacterial profile and antibiotic resistance among cancer patients with urinary tract infection in a national tertiary cancer hospital of Nepal. Tropical medicine and infectious disease. 2021;6(2):49.
24. Al-naimi IA, Abbas MA. Research article recurrent urinary tract infections in Iraqi leukemic patients. International Journal. 2016;4(3):1433-1439.
25. Islam MR, Akhter F, Khatun N, Rahman M. Bacterial Causes of Infections and Their Antimicrobial Sensitivity Pattern in Cancer Patients; c2021.
26. Kukanur S, Meundi M, Bajaj A, Kotigadde S. Co-relation between virulence factors and antibiotic resistance of E. coli, with special reference to uropathogenic E. coli. Medicine. 2015;17:32-6.
27. Pandit R, Awal B, Shrestha SS, Joshi G, Rijal BP, Parajuli NP. 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; c2020.
28. Löhr IH. Extended-spectrum β-lactamase producing Klebsiella pneumoniae. A neonatal intensive care unit outbreak, long-term colonization in children and plasmid characteristics; c2014.
29. Saleh KJ, Alakiu DM, Adam AS. Search for *E. coli* o157:h7 in stool samples from diahrreagenic patients attending dutsin-ma general hospital, Katsina state, north-western Nigeria. Int. J Biol. Sci. 2021;3(1):34-40.
DOI: 10.33545/26649926.2021.v3.i1a.27
30. Belete MA. Bacterila Profile and ESBL Screening of Urinary Tract Infection Among Asymptomatic and Symptomatic Pregnant Women Attending Antenatal Care of Northeastern Ethiopia Region. Infection and Drug Resistance. 2020;13:2579-2592.
31. Pandit R, Awal B, Shrestha SS, Joshi G, Rijal BP, Parajuli NP. 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; c2020.
32. Jena J, Sahoo RK, Debata NK, Subudhi E. 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. 2017;7(4):1-7.
33. Huang WC, Wong MY, Wang SH, Hashimoto M, Lin MH, Lee MF, *et al*. The Ferric Citrate Uptake System Encoded in a Novel bla CTX–M–3-and bla TEM–1-Harboring Conjugative Plasmid Contributes to the Virulence of Escherichia coli. Frontiers in microbiology. 2021;12:667782.
34. Dandachi I, Chaddad A, Hanna J, Matta J, Daoud Z. Understanding the Epidemiology of Multi-Drug Resistant Gram-Negative Bacilli in the Middle East Using a One Health Approach. Front Microbiol; c2019.
35. Rahim AA, Ahmadissa SM, Muhamad LR, Hama Soor TA. Antibiotic resistance: Current global issue and future challenges. Microbial Biosystems. 2021;5(2):29-68.
36. Hassuna NA, Khairalla AS, Farahat EM, Hammad AM, Abdel-Fattah M. Molecular characterization of Extended-spectrum β lactamaseproducing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. Scientific Reports. 2020;10(1):1-8.