Annals of R.S.C.B., ISSN:1583-6258, Vol. 28, Issue 1, 2024, Pages. 343-350

Received 30 July 2024; Accepted 15 August 2024

Phenotypic and molecular identification of bla_{OXA} ESBLs gene in Escherichia coli isolates from UTIs patients in the 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.**

***Corresponding author: Saad S. Mahdi Al-Amara; Tel: +96 7730324090; E-mail:** saad.mahdi@uobasrah.edu.iq

Abstract

The emergence of antibiotic-resistant microbes, including urinary tract infections, poses a significant threat to public health, making treatment increasingly challenging. Between 5th January to 22nd February, current study relied on 200 urine samples were collected from patients at Al Sadr Teaching Hospital in Al-Basrah Province, Iraq, who were suffering from urinary tract infections (UTIs). A result of current study found that 71 (35.5%) of urine samples from urinary tract infections patients showed positive bacterial growth. The growth was distributed to 47 (66.2%) Escherichia coli and 24 (33.8%) of other Gram-negative species. The diagnostic gene 16S rDNA by PCR method the results showed that all 47(100%) E. coli isolates. Furthermore,the double-disc approximation method (DAM) showed positive results in 44 (93.6%) E.coli isolates that produced ESBLs. While double-disc synergy test (DDST) showed positive results in 13(27.7%) of E.coli isolates. The study also detected Extended-spectrum β-lactamses (ESBLs) genes by PCR, amplifying the bla_{OXA} gene in E.coli isolates. The amplified genes' bands were characterized at approximately (619 bp) for bla_{OXA} and compared to the standard molecular DNA ladder at 2000 bp. The results showed that all 47(100%) E.coli isolates gave positive results for the bla_{OXA} gene.

Keywords: E.coli , ESBLs, bla_{OXA} gene.

Introduction:

The emergence of antibiotic-resistant microbes, including urinary tract infections, poses a significant threat to public health, making treatment increasingly challenging [1,2]. Healthcare-related urinary tract infections (UTIs) are prevalent and significantly impact hospitalized patients' mortality and morbidity rates, accounting for approximately 80% of these infections [3]. Urinary tract infections (UTIs) are primarily caused by Escherichia coli, and the emergence of ESBL-producing E. coli has significantly impacted UTIs management. ESBLs hydrolyze β-lactam antibiotics like penicillin's, cephalosporins, and monobactams, rendering them inactive. Large plasmids with additional antimicrobial resistance genes host ESBLs, limiting therapy choices [4,5].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 28, Issue 1, 2024, Pages. 343-350

Received 30 July 2024; Accepted 15 August 2024

Gram-negative bacteria have the ability to manufacture β-lactamases with an exceptionally broad extent, known as extended-spectrum β-lactamases (ESBLs). They mostly belong to the Enterobacteriaceae family. bla_{TEM-1}, bla_{TEM-2}, and bla_{SHV-1} β-lactamases are the sources of ESBLs; they were initially found in Western Europe and have been reported since 1980–1990[6,7]. bla_{TEM}, bla_{SHV}, bla_{CTXM}, bla_{PER}, bla_{VEB}, bla_{GES}, bla_{BES}, bla_{TLA}, and bla_{OXA} are among the nine unique structural and evolutionary families based on amino acid sequence comparisons that include the current total of over 350 different natural ESBL variations [8,9]. Extended-spectrum β-lactamase-producing strains (ESBLs) are a result of the extensive and frequently overuse of antibiotics such as β-lactams in the treatment of bacterial illnesses such as urinary tract infections (UTIs) [10,11].

A class of enzymes known as ESBLs cleaves the β-lactam ring of β-lactam antibiotics, including monobactams, broad-spectrum oxyimino cephalosporins, and penicillins, making them inactive against bacteria that can produce ESBLs[12,13]. The bla_{TEM} and bla_{SHV},type β-lactamases, bla_{CTX-M} type β-lactamases, and bla_{OXA} type β-lactamases comprise the biggest categories of ESBLs. These groups can be based on molecular homology or functional features, which is important for clinical settings since it considers substrate enzyme specificity[14]. Current study aimed to determine the frequency of ESBL-producing Gram-negative bacteria, and the molecular identification of ESBL gene bla_{OXA} in E. coli isolate among UTIs patients , in Al-Basrah province ,Iraq.

Materials and Methods:

-Collection of specimens:

Two hundred urine samples from patients with urinary tract infections (UTIs) at Al Sadr teaching hospital in Al-Basrah province, Iraq, were collected between 5th January to 22nd February.

-Isolation and identification

According to [15,16], the samples were cultivated for 24h at 37^{o}C on nutrient broth, and the samples that gave positive results were cultivated on MacConkey agar, Eosin Methylene Blue agar (EMB), and Hi-chrome^{TM} E. coli agar for isolates and identity of E. coli isolates.

- DNA extraction

Genomic DNA extracted kit (Genomic DNA Extraction Kit,Geneaid, Taiwan) was used for extracted genomic DNA from isolates according to kit protocol.

- Detection of 16S rDNA

The 16S rDNA amplification of the extracted **g**enomic DNA, which was performed by PCR using a specific primer that was approximately 585 bp in length, the results were compared using a standard molecular DNA ladder (2000 bp) [17].

- Extended Spectrum β-lactamase (ESBL) identification

-Double disk synergy test (DDST)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 28, Issue 1, 2024, Pages. 343-350

Received 30 July 2024; Accepted 15 August 2024

The CLSI recommended test used Mueller-Hinton agar plates to cultivate E.coli isolates for extended spectrum β-Lactamase (ESBL) enzyme production. Synergy between third generation cephalosporin 30µg disk and Amoxicillin- Clavulanate (20µg/10µg) antibiotics was observed, and isolates with inhibition zones towards Amoxicillin-Clvulanate were considered positive[18]**.**

-Double disk approximation method (DAM)

Four third-generation cephalosporin and monobactam antibiotic discs, as well as Amoxicillin-Clvulanate discs, were utilized in the investigation according to[18,19].

-Detection of ESBLs Genes

The specific primers that were utilized for amplified bla_{OXA} gene by PCR was approximately (619 bp) in length according to [20]. Standard molecular DNA ladder (2000 bp) was used compare the PCR results.

Results:

The study collected 200 samples from urinary tract infections (UTIs)patients from 5th January to 22nd February. 71 (35.5%) samples showed positive growth on MacConkey agar, eosin methylene blue (EMB), and HiChrome^{TM} E. coli agar. Bacterial growth was distributed to 47 (66.2%) E. coli and 24 (33.8%) other Gram-negative species. 129 (64.5%) samples showed negative results for growth. The study found significant differences ($P<0.01$) between E.coli isolates compared to other Gram-negative isolates by using ANOVA test (one way).Additionally, all 47(100%) E. coli isolates gave a positive result for the detection of 16S rDNA in molecular weights approximately 585 bp figure (1), according to the results of molecular diagnostics using the PCR technique, which depends on the diagnostic gene 16S rDNA.

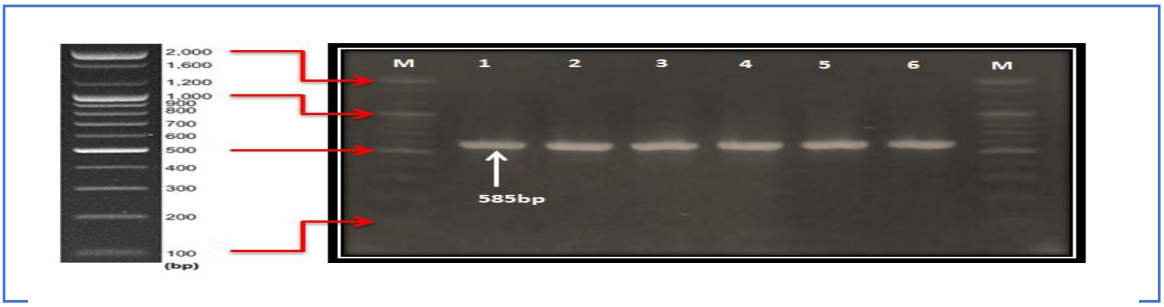

Figure (1): Agarose electrophoresis patterns show PCR amplified products of 16S rDNA. Lane1:(2000 bp DNA ladder), Lane:(no. 1-6) 16S rDNA band of E.coli isolates . using 1% agarose gel, 70V, 1h.

The study found that 44(93.6%) of E.coli isolates produced extended-spectrum β-lactamases (ESBLs) using the double-disc approximation method (DAM), while 13(27.7%) showed positive results using the double-disc synergy test (DDST). However, 3(6.4%) and 34(72.3%) showed negative results using the (DAM) and (DDST) respectively.PCR was used to amplify the bla_{OXA} gene in E.coli isolates. The amplified genes' bands were characterized

Annals of R.S.C.B., ISSN:1583-6258, Vol. 28, Issue 1, 2024, Pages. 343-350

Received 30 July 2024; Accepted 15 August 2024

approximately at (619 bp), 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_{OXA} gene as in figure (2).

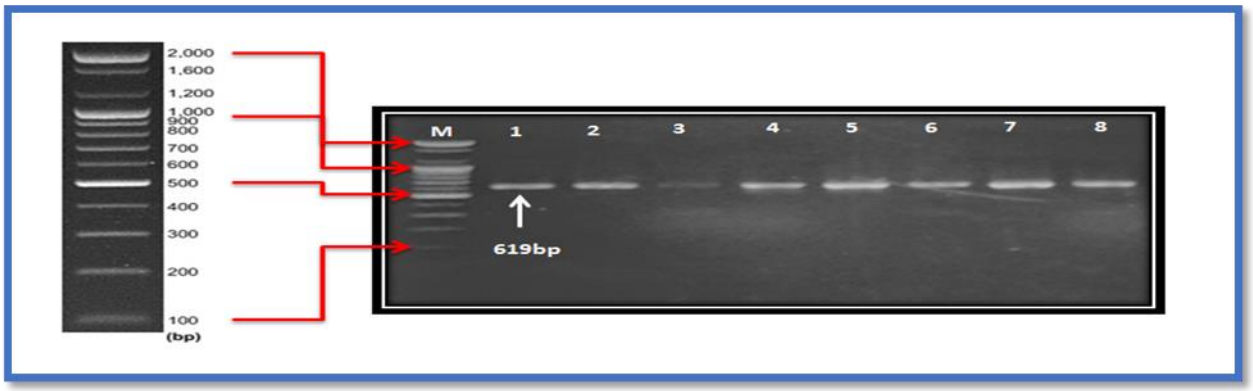

Figure 2: Agarose electrophoresis patterns of bla_{OXA} gene PCR amplified products. Lane 1:(2000 bp DNA ladder), Lane:(no. 2-8) bla_{OXA} gene bands of E.coli isolates, using 2% agarose gel, 70V, 1h.

Discussion:

According to an investigation study done in Baghdad, Iraq, E. coli is the most frequently identified bacterial in urinary tract infections (UTIs) [21]. Also E. coli was the most common cause of (UTIs) infections in Duhok, Iraq [22]. E. coli was also identified as the common costive agent of UTIs by investigations carried out in Al-Basrah [23] and Zakho, Iraq [24]. Antibiotic resistance spreads due to healthcare behaviors, environmental policies, and ineffective infection control and hygiene practices [25] The presence of ESBL-producing E. coli isolates in Iraq remains unclear, but high prevalence of community-acquired ESBL-producing isolates has been reported in Europe, Asia, and the USA.

The current study's findings reveal that the synthesis of ESBLs is consistent with previous investigations carried out through the studies of [26, 27, 28, 29, & 30]. While findings from double-disc approximation techniques (DAM) [32, 33, and 34] the current study, which identified the producer of ESBLs using the double-disc synergy test (DDST), agrees with findings from other studies done in Nepal and India [31, 32]. The emergence of antibiotic resistance might be multifactorial, but it is largely believed to be caused mainly by human activity and increased antibiotic usage for human health, animal health and food production [36]. E. coli strains have evolved to resist major classes of antibiotics such as β-lactams, quinolones, aminoglycosides, sulfonamides and fosfomycin. AmpC-producing E. coli strains are dominant in gut colonization of both animals and humans and environmental contamination in developing countries [36]

Through 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_{OXA} gene.

Received 30 July 2024; Accepted 15 August 2024

Statistical analysis revealed no significant differences P>0.05 between the isolates that tested positive or negative for the bla_{OXA} gene. present study agreed with [35,36&37]. E. coli possesses virulence and resistance genes that are highly contagious among various types of bacteria [38,39]. Conjugated plasmids are an important horizontally gene transferee (HGT) agent that efficiently spreads these genes. Everyone is really at risk from these plasmids. Iraq is a developing country in the Middle East that is facing multidrug resistance as a result of improper use of antibiotics in humans and animals, inadequate hygiene, insufficient control mechanisms, and restricted sales of antibiotics in pharmacies. Over the last 20 years, Iraq has endured both military and economic crises [40,41,42].

Conclusions: Gram-negative bacteria, particularly those that produce ESBL, constitute a concern to public health, E. coli isolates with the bla_{OXA} gene associated to medicine resistance show a threat to global healthcare.

Reference

1. NM Ayat, Ghaly MF, Zahira MF, Amer MM, Yosra EM.(2010) Antibacterial activities of plant extracts combined withantibiotic drugs on clinical Escherichia coli isolated from urinary tract infection. In: Proceeding of Fifth Scientific Environmental Conference; 2010; Ash Sharqiyah, Egypt: Zagazig University.
2. El Hassan MME.(2013) Prevalence of TEM, SHV and CTXM genes inEscherichia coli and Klebsiella spp. urinary isolates from Sudan with confirmed ESBL phenotype. Life Sci J.10: 191-195.
3. Jean SS, Coombs G, Ling T, et al. (2016)Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2010-2013. Int J Antimicrob Agents 2016;47(04):328–334
4. Foxman B. (2002)Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002; 113: 5–13.
5. Petty NK, Ben Zakour NL, Stanton-Cook M et al.(2014) Global dissemination of a multidrug resistant Escherichia coli clone. Proc Natl Acad Sci USA 2014; 111: 5694–9.
6. Sharma J, Sharma M, Ray P. (2010)Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res. 132:332–336.
7. Miao Z, Li S, Wang L, Song W, Zhou Y.(2017) Antimicrobial resistance and molecular epidemiology of esbl-producing Escherichia Coli isolated from outpatients in town hospitals of shandong province, China. Front Microbiol.8:63.
8. Shahid M, Singh A, Sobia F, et al.(2011) bla(CTX-M), bla(TEM), and bla(SHV) in Enterobacteriaceae from North-Indian tertiary hospital: high occurrence of combination genes. Asian Pac J Trop Med. 4(2):101–105.
9. Bajpai T, Pandey M, Varma M, Bhatambare GS. (2017) Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med.;7(1):12-16.

10. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA (2015)Antibiotic resistance and extended spectrum beta-lactamases:Types, epidemiology and treatment. Saudi J Biol Sci 22: 90-101.
11. poda DS, Ajayi A, Somda M, Traore O, Guessennd N,Ouattara AS, Sangare L, Traore AS, Dosso M (2018)Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. BMC Res Notes 11: 471.
12. Rawat D, Nair D (2010) Extended-spectrum beta-lactamases inGram-negative Bacteria. J Glob Infect Dis 2: 263-274
13. Livermore DM, Day M, Cleary P, Hopkins KL, Toleman MA,Wareham DW, Wiuff C, Doumith M, Woodford N (2019).OXA-1 beta-lactamase and non-susceptibility to penicillin/beta-lactamase inhibitor combinations among ESBL-producing Escherichia coli. J Antimicrob Chemother 74: 326-333.
14. Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. (2019)Distribution of bla CTX - M, bla TEM, bla SHV and bla OXA genes in extendedspectrum-β-lactamase-producing clinical isolates: a three-year multi-center study from Lahore, Pakistan. Antimicrob ResistInfect Control,8(01):80
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 of E. 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. Sharma, M., Pathak, S., & Srivastava, P. (2013). 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, 7(10), 2173.
21. 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.
22. 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.

23. 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.
24. 24.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.
25. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al.(2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect.18(3):268–81.
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. Ali, 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. 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.
32. 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).
33. 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.
34. 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.

35. 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.
36. 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.
37. 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.
38. Jena J, Sahoo RK, Debata NK, Subudhi E. Prevalence of TEM, SHV, and CTX-M genes of extendedspectrum β-lactamase-producing Escherichia coli strains isolated from urinary tract infections in adults. 3 Biotech. 2017;7(4):1-7.
39. 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.
40. 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.
41. Rahim AA, Ahmadissa SM, Muhamad LR, Hama Soor TA. Antibiotic resistance: Current global issue and future challenges. Microbial Biosystems. 2021;5(2):29-68.
42. Hardany ,M. J. , Al-Abdullah, A. A. , Al-Amara, S. S. , Makki, H. M.(2020). Detection ESBL enzymes in Gram-negative bacteria. Journal of Basrah Researches (Sciences). Volume 45, (2): 54-59.