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Detection and antibiotic resistance genes of diarrheagenic Escherichia coli from Pathotypes in Children Under five years

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Abstract

Escherichia coli exists both as a benign resident in the intestines of many mammals and as a potential harmful pathogen. this study aimed to investigate about many virulence genes can be acquired by commensal E. coli to become pathogenic one and specifically causes diarrhea. 100 stool samples were taken from healthy children as a control group, and 500 stool samples were taken from children under five who had diarrhea. *E. coli* was isolated using standard techniques from every sample, then E. coli isolates from both patients and controlled has molecularly genotyped to characterize commensal ones, the investigation about *Stx1, Stx2* using multiplex PCR. The results showed that 150(30%) of the isolated bacteria from diarrhea samples were *E.coli* ,122 of them were commensal ones. The results also showed that Stx1 gene present in all commensal isolated *E.coli* from patients ,while Stx2 was present in 118samples only. These genes can be easily transferred to commensal *E. coli* via horizontal gene transfer under many serious conditions such as malnutrition , abuse of antimicrobial agents and poor hygiene. Beside disease these transferred genes can also change both core genome and the pan-genome in commensal bacteria.

Keywords: Diarrheagenic, E. *coli*, multiplex PCR, *ChuA* gene, *Yja A* gene, *stx1*and *stx2* genes

Introduction

The most common aerobic bacterium in humans' and other mammals' gut microbiota is *Escherichia coli*. [1]. Despite being widespread, it can lead to a number of illnesses, such as extra-intestinal and diarrheal conditions [2]. Since 2000, *E. coli* has been one of the most prevalent bacteria that contributes to the development and dissemination of antibiotic resistance factors, including genes that code for CTX-M and other extended spectrum β-lactamases [3]. Due to its extensive host range and the severity of the diseases it can cause, combating resistant pathogenic *E. coli* is a global health priority [4]. Studying the genetic structure of gut *E. coli* populations is crucial, as factors like antibiotic treatments drive the emergence and spread of resistance genes [5]. The seven primary phylogenetic groups of *E. coli* species are A, B1, B2, C, D, E, and F. The effectiveness of these groups in colonizing the gut and their capacity to produce extra-intestinal or intestinal illnesses vary [6]. The high virulence of pathogenic strains in phylogenetic group B2 may be linked to their superior gut colonization capability [7]. *E. coli* serves as an important marker for monitoring the emergence of resistant pathogens in the gut microbiota due to its dual role as both a commensal and a pathogen [8]. Humans have a close relationship with the microbes in their bodies, particularly in the gut, which provides an ideal environment for these bacteria to thrive. The gut microbiota is crucial for the development and maturation of the gut and the differentiation of the immune system [9]. It significantly influences both physical and mental development in children [10]. An imbalance in the gut microbiota, known as symbiosis, can contribute to various childhood disorders, both within and outside the gastrointestinal tract. Probiotics might help manage these disorders to varying extents [11]. However, more research is needed to confirm their effectiveness, determine the appropriate probiotic for each condition, establish the correct dosage, and ensure safety [12]. Commensal *Escherichia coli* (*E. coli*) can become pathogenic through several mechanisms [13]. These processes often involve the acquisition of genetic elements that encode virulence factors, which enhance the bacteria's ability to cause disease, such as horizontal gene transfer, acquisition

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of mobile genetic elements like a plasmids, transposons or pathogenicity islands [14] and mutations which usually increase the expression of existing virulence genes [15]. This study's objective is to determine and detection some antimicrobial resistant genes aqueried by commensal *E. coli* that cause diarrhea in children under five years of age who experiencing acute diarrhea, we focused our efforts on describing the prevalence of DEC in Al Diwaniyah province.

Methods

Collection of Samples: Between September 2024 and March 2025, 500 stool samples from children under five years old at the city's Children Teaching Hospital were collected for the current study. 100 stool samples from healthy kids in the same age range as previously described served as the control; they had not taken any antibiotics for three months before to collection.

Stool samples cultivation: All samples were transferred as quickly as possible to the laboratories of the College of Science at Al-Qadisiyah University for the purpose of culturing them on blood agar and MacConkey media. Then the Gram-negative rods were streaked on eosin-methylene blue medium for the purpose of confirming that they were *E. coli* bacteria.

E. coli strain diagnosis: The colonies that gave a green color with a metallic shine were transferred to E.M.B. medium for the purpose of diagnosis using API20I, then antibiotic sensitivity test was done according to CLSI 2023 recommendations for *E. coli* isolated strains [16].

Molecular typing of commensal *E. coli*: Two genes, *YjaA* and *ChuA*, were used to molecularly diagnose commensal E. coli strains. Primers for these genes were created using the NCBI-Genbank database sequence (MH511180) and (MH511176), which have wave lengths of 219 and 279 bp, respectively. Primers were created using the primer design online software. and these primers, as indicated in table 1, were supplied by Scientific Researcher Co. Ltd. in Iraq.

Table 1. The primers provided for commensal *E.coli* typing

Primers	Sequence (5'-3')		Amplicon
Yja A gene	F	GAGCTGATCCGGCTTGTA	219bp
	R	AATTCAGAGGAGGGAGGC	
ChuA gene	F	GAGTTGATCGGCCTTGTA	279bp
	R	AATCAAGAGAGGGCAGGT	

The bacterial DNA extracted depending on gram-negative bacteria protocol by using bacterial DNA extraction kit provided from Trans company\ China. then the extracted DNA was genotyped using multiplex polymerase chain reaction (PCR).

The study also looked into the existence of some genes called AMR (Anti-Microbial Resistance) genes, which induce diarrhea and multiple antibiotic resistance. The multiplex polymerase chain reaction (PCR) was used to examine the presence of three genes: *Stx1, Stx2*.The information from [17] was used to design the primers that are shown in Table 2.

Table 2 .The oligonucleotides primers sequence used in PCR

Primers	Sequence (5'-3')		Amplicon
Stx1	F	ACACTGGATGATCTCAGTGG	614bp
	R	CTGAATCCCCCTCCATTATG	
Stx2	F	CCATGACAACGGACAGCAGTT	779bp
	R	CCTGTCAACTGAGCAGCACTTTG	

Results

A total of **500** samples have been collected, only 150 (30 %) isolates were belonging to *E. coli* Figure (1a) , in which 10% of them were isolated from1-2 years, 20 % were isolated from 2-3 years while 30 % and 40% were isolated from 3-4 years and 4-5 years respectively.

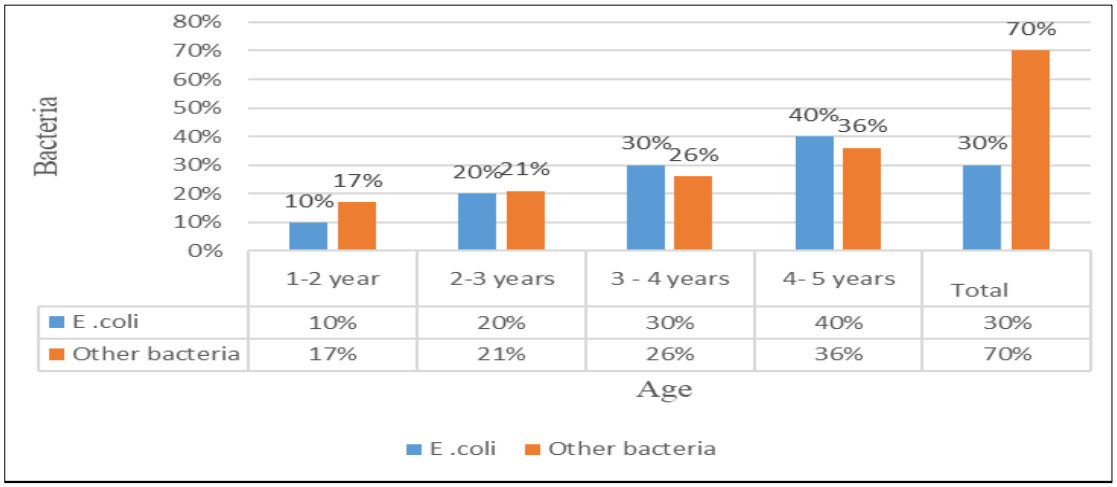


Figure 1a. Showing the number of isolated *E. coli* from diarrhea patients

At first, the bacteria were isolated and diagnosed using traditional methods from the stool samples of both children with diarrhea and healthy ones . Five hundred feces samples from kids were gathered. returning to the governorate's hospitals due to diarrhea during the study period, and 100 stool samples from children in good health were gathered. that were used as control, Figure (1b).

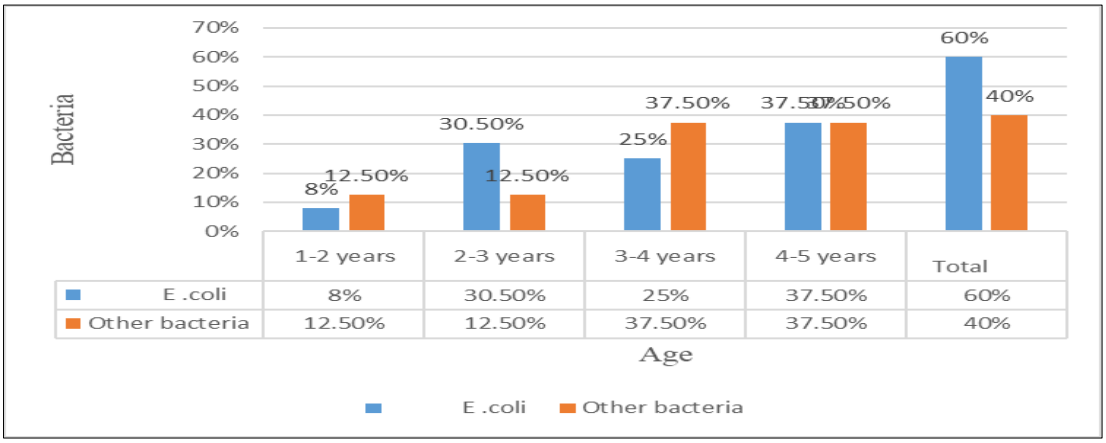


Figure 1b: Showing the number of isolated *E. coli* from control

Antibiotic sensitivity Results

The antibiotic sensitivity was testing for all *E.coli* isolates using the disk propagation kirby Bauer method. based on the Clinical Laboratory Standard Institute (CLSI 2023) recommendations [18]. Tthe table four below describe the results of antibiotics sensitivity testing for *E.coli* that isolated from diarrhea patients and control.

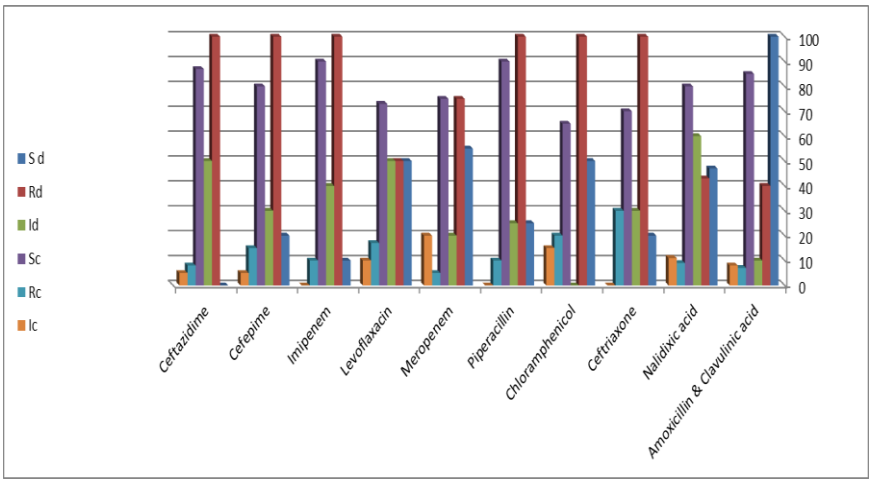


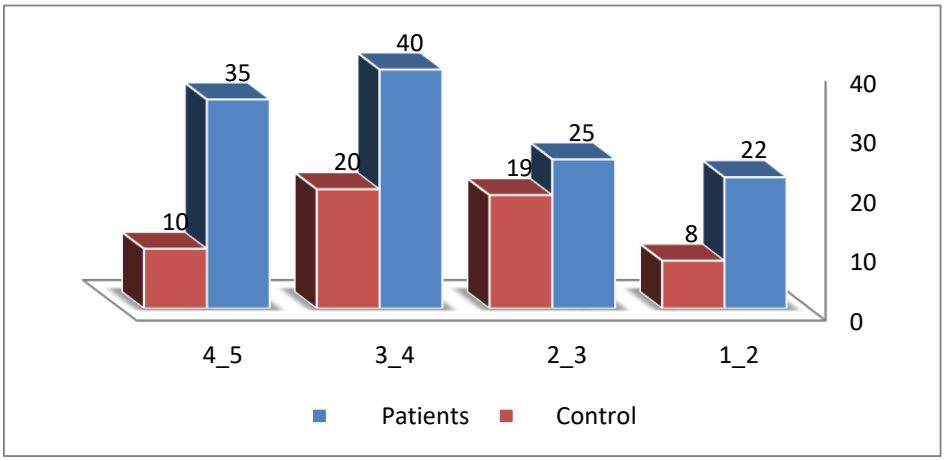
Figure 2. Describe of antibiotics sensitivity testing for *E.coli* that isolated from diarrhea patients and control.

* Sd: Susceptible diarrhea ; Rd: Resistant diarrhea; Id: Intermediate diarrhea
*Sc: Susceptible Control ; Rc: Resistant control; Ic: Intermediate control

Molecular typing of commensal *E. coli* and investigation about AMR genes results :

The previous investigation using multiplex PCR for typing commensal *E .coli* in children under five years with diarrhea and also for detection some AMR genes in the commensal *E .coli,* these genes usually isolated from pathogenic *E .coli* and mainly responsible for many virulence characteristics such as adherence and antibiotics resistant [19]. The table below shows the numbers of commensal *E .coli* in the stool samples of both patients and controls.

Figure3. Distribution of Commensal *E. coli* in diarrhea patients and control



The AMR genes that were examined in the earlier investigation were *s\Stx1* and *Stx2*.The differentiation of these genes in commensal *E. coli* in both patients and controls was displayed in Figure4.

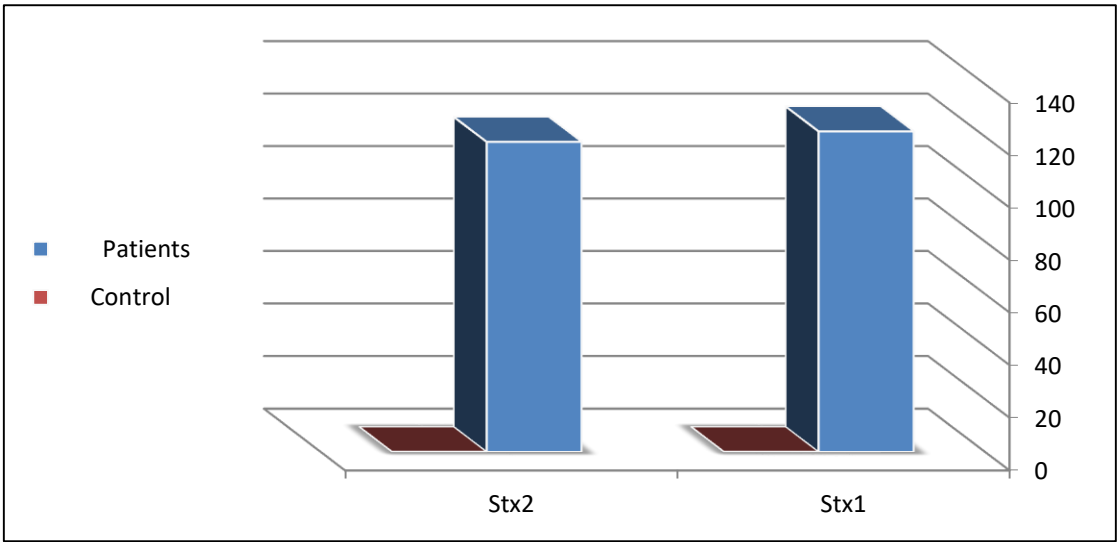


Figure 4. Distribution of AMR genes in commensal *E. coli* in both diarrhea patients and control

Discussion

According to the method used in this study, it was revealed that 30% of children with diarrhea were infected with DEC. Our results are nearly similar with previous reports regarding the prevalence of DEC infection in children less than 5 years old from Bangladesh [20], Iran, [21,22] respectively, and Iraq [23]. However, the reported frequencies of DEC are variable in other studies with reports showing frequencies in Syria [24], and in southeastern China [25]. The observed contrasts between the present study and previous reports could be due to variations in sample size, culturing techniques, culture media, and specific incubation conditions.

The prevalence of DEC among children aged 1-2 year, aged 2-3 years, 3-4 years and aged 4–5 years old were 10 , 20, 30 and 40% respectively. These findings agree with [26,27], but dont agree with [28].

All DEC isolates were multi drug resistant to antibiotics, showing resistance to four classes of antibiotics used. High rates of resistance were recorded against antibiotics to Ceftriaxone, Ceftazidime, Chloramphenicol, Piperacillin, Imipenem , and Cefepime because widely used of these antibiotics and widely used in developing countries to treat diarrhea due to their low cost and availability, which leads to increased resistance to these antibiotics.While lower resistance rates were observed for to Amoxicillin & Clavulinic acid , Nalidixic acid , Levoflaxacin and Meropenem in this study.

The highest resistance to antibiotics was (100%) to Ceftriaxone, Ceftazidime, Chloramphenicol , Piperacillin, Imipenem , and Cefepime agreement with another study had been reported high resistance in studies [29, 30, 31 ,32,33,34] .Ciprofloxacin was one of the most active antimicrobial agent which currently recommended to treated diarrhea in children [35]. Worldwide prevalence of high resistance in DEC could be attributed to the inappropriate and wide use of different antibiotics to treat infection in children of a young age. The high incidence of antibiotic resistant isolates of DEC may be due to the widespread use of antibiotics. The transfer of resistance genes that may occur between species could lead to the construction of diverse resistance to usual antibiotics [36].

Meropenem belong to carbapenem antibiotic binds to different penicillin binding protein. It is from the most active drug against gram negative bacteria , the sensitivity of Enteroaggregative E.coli to meropenem was (55%) agreement with [37 ,38]. *E. coli* exhibited a (40%) resistance to amoxicillin/clavulanic acid, agreement with another studies as done by [39,40 ,41].The higher resistance of *E. coli* to amoxicillin/clavulanic acid among children with diarrhea . One explanation is that the broad use of a single antibiotic or a specific class of antibiotics can foster the development of bacterial resistance.

Our study for resistance to Nalidixic acid and Levoflaxacin (43, 50%) respectively, agreement with [42, 43]. With an increase in resistant strains occurring globally, antibiotic resistance poses a serious threat to both public health and medicine. Despite this, recent research has shown that commensal resistance can also help treat human infections with antibiotics by encouraging the ongoing ecological suppression of pathogens (18).but cannot dispute that all types of antibiotic resistance, including those found in non-pathogenic bacteria, are regarded as risky. Because commensal bacteria can harbor resistance genes that could be passed on to pathogens or they themselves could eventually cause opportunistic infections, resistance in these organisms is very dangerous.

In accordance with Figure 3, the percentage was computed in relation to the quantity of *E. coli* isolates from the samples. It is clear from multiplex PCR results for commensal *E. coli* identification, which depended on both the *Yja A* and *ChuA* genes, that patients with diarrhea have a larger percentage of commensal *E. coli* than controls in the same age group. The *YjaA* gene's precise role in *E. coli* is not well understood. It is frequently employed as a genetic marker in research, especially when identifying and differentiating strains of *E. coli*. It is employed to divide E. coli into various groups in molecular epidemiological and phylogenetic investigations. It is one of several markers used to track the origins and evolutionary relationships of E. coli strains using multi-locus sequence typing (MLST) and other genotyping techniques [44]. An outer membrane heme receptor protein, which is encoded by the *ChuA* gene, is implicated in the use of heme as an iron source [45]. Particularly in settings where iron is scarce, like the human body, this activity is essential for iron uptake. When examining the genetic diversity, toxicity, and ecological adaptability of *E. coli*, both *YjaA* and *ChuA* are useful. They aid in comprehending how distinct strains endure, spread, and infect people in diverse settings.[46].

The primary goal of the current study was to investigate AMR genes. It can be characterized as the genes that cause resistance to antibiotics. Actually, pathogen genetic changes over time cause AMR, which is a natural process. Its development and spread have been exacerbated by human activities, including the misuse and abuse of antibiotics for the treatment, prevention, or control of diseases in humans, animals, and plants [46].The AMR genes that were examined in the earlier investigation were *Stx1* and *Stx2*.The differentiation of these genes in commensal E. coli in both patients and controls .The Shiga toxin is the most important toxin associated with the pathogenicity of E. coli O157.The high prevalence *Stx1* (100%), *Stx2*(96.7%), These results were compatible with the study [47 ,48 ,49].

Conclusions

This study demonstrated the importance of pathotypes E. coli in diarrhea in children. To prevent and treat infectious diarrhea in children under five, an epidemiologic surveillance specifically for DEC might be helpful.

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References

1. Martinson, J. N., & Walk, S. T. (2020). *Escherichia coli* residency in the gut of healthy human adults. EcoSal plus, 9(1), 10-1128.
2. Martinson, J. N., Pinkham, N. V., Peters, G. W., Cho, H., Heng, J., Rauch, M., ... & Walk, S. T. (2019). Rethinking gut microbiome residency and the Enterobacteriaceae in healthy human adults. The ISME journal, 13(9), 2306-2318.
3. Foster-Nyarko, E., & Pallen, M. J. (2022). The microbial ecology of Escherichia coli in the vertebrate gut. FEMS microbiology reviews, 46(3), fuac008.
4. Basavaraju, M., & Gunashree, B. S. (2022). Escherichia coli: an overview of main characteristics. Escherichia coli-Old and New Insights.
5. Christofi, T., Panayidou, S., Dieronitou, I., Michael, C., & Apidianakis, Y. (2019). Metabolic output defines *Escherichia coli* as a health-promoting microbe against intestinal Pseudomonas aeruginosa. Scientific reports, 9(1), 14463.
6. Panayidou, S., & Apidianakis, Y. (2017). *Pseudomonas aeruginosa*. In Laboratory Models for Foodborne Infections (pp. 373-389). CRC Press.
7. Tawfick, M. M., Elshamy, A. A., Mohamed, K. T., & El Menofy, N. G. (2022). Gut commensal *Escherichia coli*, a high-risk reservoir of transferable plasmid-mediated antimicrobial resistance traits. Infection and Drug Resistance, 1077-1091.
8. Saeed, N. K., Al-Beltagi, M., Bediwy, A. S., El-Sawaf, Y., & Toema, O. (2022). Gut microbiota in various childhood disorders: Implication and indications. World Journal of Gastroenterology, 28(18), 1875.
9. Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. Biochemical journal, 474(11), 1823-1836.
10. Collado, M. C., & Segata, N. (2020). Initial exploration of in utero microbial colonization. Nature medicine, 26(4), 469-470.
11. Suez, J., Zmora, N., Segal, E., & Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. Nature medicine, 25(5), 716-729.
12. Ramos, S., Silva, V., Dapkevicius, M. D. L. E., Caniça, M., Tejedor-Junco, M. T., Igrejas, G., & Poeta, P. (2020). *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. Animals, 10(12), 2239.
13. National Research Council, Division on Earth, Life Studies, Board on Life Sciences, & Committee on Scientific Milestones for the Development of a Gene Sequence-Based Classification System for the Oversight of Select Agents. (2010). Sequence-based classification of select agents: a brighter line.
14. Sobota, M., Rodilla Ramirez, P. N., Cambré, A., Rocker, A., Mortier, J., Gervais, T., ... & Diard, M. (2022). The expression of virulence genes increases membrane permeability and sensitivity to envelope stress in *Salmonella Typhimurium*. Plos Biology, 20(4), e3001608.
15. Rai, S., Dash, D., & Agarwal, N. (2023). Introducing the new face of CLSI M100 in 2023: An explanatory review. Indian Journal of Medical Microbiology, 46, 100432.
16. Alsaadi, Z. H., Tarish, A. H., & Saeed, E. A. (2018). Multiplex PCR rapid and sensitive screening method for detection of local strains of Escherichia coli O157: H7 in Hilla city. Biochem. Cell. Arch, 18(1), 31-36.

17. Wollein Waldetoft, K., Sundius, S., Kuske, R., & Brown, S. P. (2023). Defining the benefits of antibiotic resistance in commensals and the scope for resistance optimization. MBio, 14(1), e01349-22.
18. Abbasi, P., Kargar, M., Doosti, A., Mardaneh, J., Ghorbani-Dalini, S., & Dehyadegari, M. A. (2014). Characterization of Shiga-toxin producing E. coli (STEC) and enteropathogenic E. coli (EPEC) using multiplex Real-Time PCR assays for stx1, stx2, eaeA. Iranian journal of microbiology, 6(3), 169.
19. Dadi, B. R., Abebe, T., Zhang, L., Mihret, A., Abebe, W., & Amogne, W. (2020). Distribution of virulence genes and phylogenetics of uropathogenic Escherichia coli among urinary tract infection patients in Addis Ababa, Ethiopia. BMC infectious diseases, 20, 1-12.
20. Aminul Islam M., Heuvelink A.E., De Boer E., Sturm P.D., Beumer R.R., Zwietering M.H., et al, (2007). Shiga toxin-producing Escherichia coli isolated from patients with diarrhea in Bangladesh, J. Med. Microbiol.56 380–385. https://doi.org/10.1099/jmm.0.46916-0.
21. Broujerdi S.M., Ardakani M.R., Rezatofighi S.E. (2018). Characterization of diarrheagenic Escherichia coli strains associated with diarrhea in children, Khouzestan, Iran. J Infect Dev Ctries. Aug 31;12(8):649-656. doi: 10.3855/jidc.9538.
22. Heidary M, Momtaz H, Madani M. (2014). Characterization ofdiarrheagenic antimicrobial resistant *Escherichia coli* isolated from pediatric patients inTehran, Iran. Iran Red Crescent MedJ 16: e12329.
23. Khalil Z.K. (2015) .Isolation and identification of different diarrheagenic (DEC) Escherichia coli pathotypes from children under five years old in Baghdad. Iraqi Journal of Community MedicinE,vol 28, no 3, pp.126-132.
24. A. Homsi ,W. Al-Said1, Z.Tahan.(2023).Detection of Some Virulence Genes in Diarrheagenic Escherichia Coli Pathotypes in Children Under Five Years in Aleppo City . Al-Mustansiriyah Journal of Science Volume 34, Issue DOI: https://doi.org/10.23851/mjs.v34i4.1359.
25. Chen Y, Chen X, Zheng S, Yu F, Kong H, Yang Q, Cui D,Chen N, Lou B, Li X, Tian L, Yang X, Xie G, Dong Y, Qin Z,Han D, Wang Y, Zhang W, Tang YW, Li L.(2014). Serotypes,genotypes an antimicrobial resistance patterns of human diarrhoeagenic Escherichia coli isolates circulating in southeastern China. Clin Microbiol Infect 20: 52-58.
26. - Khairy R. M. M., Fathy Z.A., Mahrous D.M., Mohamed E. S. and Abdelrahim S. S. (2020). Prevalence, phylogeny, and antimicrobial resistance of Escherichia coli pathotypes isolated from children less than 5 years old with community acquired- diarrhea in Upper Egypt. Khairy et al. BMC Infectious Diseases , 20:908 . https://doi.org/10.1186/s12879-020-05664-6.
27. Zhou Y, Zhu X, Hou H, et al. (2018).Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: a hospital-based study. BMC Infect Dis. 18:63.
28. Hasan H.K. , Yassin N.A., Eassa S.H.(2020). Bacteriolgical and molecular characterization of diarrheagenic Escherichia coli pathotypes from children in Duhok city ,Iraq. Science Journal of University of Zakho 8(2), 52-57.
29. Sakhi R.(2019). Isolation of Escherichia Coli from Diarrhea and Test Their Pathogensity and Susceptibility Pattern for Antibiotic. Journal of College of Education for Pure Science ;9(1):43–48.
30. Al Hilali S.A., Almohana A. M. (2011) .Occurrence and molecular characterization of enteropathogenic Escherichia coli serotypes isolated from children with diarrhoea in Najaf, Iraq. Indian J Med Microbiol.Oct-Dec;29(4):383-8. doi: 10.4103/0255-0857.90171.

31. Amin M., Sirous M.(2018). Antibiotic resistance pattern and molecular characterization of extendedspectrum β-lactamase producing Enteroaggregative Escherichia coli isolates in children from southwest Iran. Infection and Drug Resistance;11:1097–1104.
32. Alkhudhairy, Miaad K.1; Saki, Morteza2,3,; Seyed–Mohammadi, Sakineh2,3; Jomehzadeh, Nabi4; Khoshnood, Saeed5; Moradzadeh, Mina2; Yazdansetad, Sajjad. (2019).Integron frequency of Escherichia coli strains from patients with urinary tract infection in Southwest of Iran. Journal of Acute Disease 8(3):p 113-117,.DOI: 10.4103/2221-6189.259110.
33. Rajabnia M.,Forghani M.S.,Hasani S.,Bahadoram M.,Mohammadi M., Barahman M.(2019). Prevalence and antibiotic resistance pattern of extended spectrum beta lactamase producing Escherichia coli isolated from urinary tract infection .J Renal Inj Prev ;8(2): 78-81.doi: 10.15171/jrip.15.
34. Shatub W. T., Jafar N. A., and Melconian A. K.(2021). Detection of diarrheagenic E.coli among children under 5 years age in Tikrit city of Iraq by using single multiplex PCR technique . Plant Archives Vol. 21, Supplement 1, pp. 1230-1237.
35. Ayatollahi, J.; Shahcheraghi, S.H.; Akhondi, R. and Soluti, S. (2013). Antibiotic Resistance Patterns of *Escherichia coli* Isolated from Children in Shahid Sadoughi Hospital of Yazd. Iranian journal of pediatric hematology and oncology, 3(2): 78–82.
36. Aslani, M.M.; Alikhani, M.Y.; Zavari, A.; Yousefi, R. and Zamani, A.R. (2011). Characterization of enteroaggregative *Escherichia coli* (EAEC) clinical isolates and their antibiotic resistance pattern. International Journal of Infectious Diseases. 15: 136– 139 .
37. Jabur S.G., Abed M.H.(2020). Genetic Survey of Enteroaggregative E.coli in Diarrheic Children under 5 years in Thi-qar governorate. Indian Journal of Forensic Medicine & Toxicology, July-September ,Vol. 14, No. 3.
38. Yasmin, S.; Karim, A.-M.; Lee, S.-H.; Zahra, R.(2022). Temporal Variation of Meropenem Resistance in *E. coli* Isolated from Sewage Water in Islamabad, Pakistan. Antibiotics 11, 635. https://doi.org/ 10.3390/antibiotics11050635.
39. Yakubov R, Vanden AM, Machamad K, Amit H, Erez N, et al. (2017).Antimicrobial resistance among uropathogens that cause childhood community-acquired urinary tract infections in central Israel. Pediatr Infect Dis J 36(1): 113-115.
40. Rosado MR, Molina AG, Velasco AL, Gloria CC, Paula VL, et al. (2022).Urinary tract infection in pediatrics: Study of uropathogens and theirresistance in a Madrid hospital. Arch Esp Urol 75(9): 791-797.
41. Hilbert S., Hedjri M., Surböck M. and Minkov M.(2024) .Escherichia Coli Resistance to Amoxicillin-Clavulanic Acid in Pediatric Urinary Tract Infections. Res Pediatr Neonatol. 8(1). RPN. 000680. DOI: 10.31031/RPN.08.000680.
42. Malekzadegan A., Rastegar E., Moradi M., Heidari H., Saraie H.S.E.(2019). Prevalence of quinolone-resistant uropathogenic Escherichia coli in a tertiary care hospital in south Iran .Infection and Drug Resistance :12 1683–1689.
43. Ali S. A., Al-Dahmoshi H. O.M. (2021).Antibiotic Resistance Profile of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Patients with Cystitis . Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, Pages. 1336 - 1347 .
44. Abu, D., Abula, T., Zewdu, T., Berhanu, M., & Sahilu, T. (2021). Asymptomatic Bacteriuria, antimicrobial susceptibility pattern and associated risk factors among pregnant women attending antenatal care in Assosa General Hospital, Western Ethiopia. BMC microbiology, 21(1), 348.

45. Sonkar, N., Banerjee, M., Gupta, S., & Ahmad, A. (2021). Asymptomatic bacteriuria among pregnant women attending tertiary care hospital in Lucknow, India. Dubai Medical Journal, 4(1), 18-25.
46. World Health Organization. (2018). Antimicrobial resistance and primary health care (No. WHO/HIS/SDS.56). World Health Organization.
47. Tula M. Y., Enabulele O. I., Ophori E.A.(2023). Occurrence and antibiotic resistance profile of shiga toxin-producing Escherichia coli (STEC) and enteropathogenic Escherichia coli (EPEC) from sources of water in Mubi Region, Adamawa State, Nigeria. Public Health Toxicol;3(3):15 https://doi.org/10.18332/pht/172303.
48. Khalil R. K.S. , Skinner C., Patfield S. and He X.(2016). Phage-mediated Shiga toxin (Stx) horizontal gene transfer and expression in non-Shiga toxigenic *Enterobacter* and *Escherichia coli* strains. Pathogens and Disease, 74, ftw037 , doi: 10.1093/femspd/ftw037.
49. Akomoneh E. A., Esemu S. N., Kfusi A. J., Ndip R. N., Ndip L. M.(2020). Prevalence and Virulence Gene Profiles of Escherichia coli O157 from Cattle Slaughtered in Buea, Cameroon . bioRxiv preprint doi: https://doi.org/10.1101/06.19.161166.