How to Cite:
Bager, A. M., & Naeem, A. K. (2022). Genotypic detection of lifA and fimH genes in Enter-Pathogenic E.coli Isolated from children suffering from diarrhea in AL-Najaf City, Iraq. *International Journal of Health Sciences*, *6*(S5), 1314–1321. https://doi.org/10.53730/ijhs.v6nS5.8865

Genotypic detection of *lifA* and *fimH* genes in Enter-Pathogenic *E.coli* Isolated from children suffering from diarrhea in AL-Najaf City, Iraq

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***Abstract*---**This study aimed to identification of genotypic characterization of *lifA* and *fimH* genes in 30 Enteropathogenic *E.coli* (EPEC) isolated from children suffering from diarrhea using Polymer Chain Reaction (PCR) technique. The results of agarose gel electrophoresis of *lifA* and *fimH* amplicons showed that 19(63%) and 25(83%) isolates were possess these gene respectively.Conclusions: There were high incidence of *fimH* and *lifA* genes in EPEC isolated from children suffering from diarrhea.

***Keywords*---**EPEC, *lifA*, *fimH*, children, diarrhea.

Introduction

Diarrhea is the second leading cause of death in children under five years of age. Diarrhea is described as a sickness that causes the passing of three or more loose stools per day or more frequently than is normal for a healthy person. Many Factors could contribute to diarrhea such nutritional factors, hygiene conditions and environmental (Tareen et al., 2022). Diarrhea affects people of all ages, although infants and children under the age of five are more susceptible to diarrheal infections than other age groups (Munhoz etal., 2021). *Escherichia coli*, *Campylobacter* spp, *Clostridium perfringens*, *Salmonella spp* and *Shigella spp*. Are among the organisms that cause infectious bacterial diarrhea (Nanok et al., 2015). *E. coli* is an important member of the microbiota of various hosts, due to its ability developed a pathos-features allowing it to adapt and live in varied conditions. So that pathogenic *E.coli* can cause a wide spectrum of human diseases (Lawal et al.,2022). In *E. coli* 80% of all strains have the capacity to express type 1 fimbriae. Arguably, the fact that these adhesive organelles are so common is indicative of important roles in the ecology of these bacteria. Fimbriae consisting of all the structural components, i.e., *fimA, fimD, fimF, fimG, and fimH*

(Bessaiah et al., 2021). It is now believed that virulence in *E. coli* is multifactorial and that certain properties are associated primarily with virulent strains. The main of this study is to identification of genotypic characterization of *lifA* and *fimH* genes in 30 Enter-pathogenic *E.coli* (EPEC) isolated from children suffering from diarrhea using Polymer Chain Reaction (PCR) technique.

Methods

Samples Collection

A total of 100 stool samples have been collected from children with acute diarrhea under 5 years old of both sex whom attended to Al-Zahraa Teaching Hospital during the period from September 2021 to Novmber2021.The stool samples were collected in special sterile containers and all information about the patient about patient were collected using a questioner. All samples were transported to the Advance Microbiological Laboratory, Department of Biology, Faculty of Education for Girls, University of Kufa for further manipulation of samples.

Ethical Approve

Samples were collected from patients after obtaining consent from them and informing them that the samples taken are for research purposes accepted methods.

Isolation and Identification of Bacterial Isolates (EPEC)

A stool sample were cultured directly on MaConkey agar for primary isolation of EPEC and incubated at 37C for 24 hr,A Lactose fermenter colonies were transferred to inoculate EMB by streaking method to confirmed primary identification of EPEC,A metallic shine colonies were purified and preserved. Microscopic examination and biochemical tests were followed up for further identification of bacterial isoltes depending on MacFaddin, (2000).

Extraction of Bacterial DNA:

Boiling Methods that describe breviously (Sambrook and Russell,2001) has been followed up for isolation of DNA from bacterial isolates.

Primers

The sequences all primers used in the polymerase chain reaction technique were explained in table 1.

Table 1: The Specific Oligonucleotides (primers) and their sequences

Genes	Oligonucleotide Sequence 5′ ⟶ 3′		Volume to yield 100 Pmol / µL	Molecular Weight of Amplicons (bp)	References
fimH	F	TGCAGAACGGATAAGCCGTGG	300	508	Johnson and Stell, (2000)
	R	GCAGTCACCTGCCCTCCGGTA			

	R	GTAGCCAACCCCCGATGAAA			
lifA	F	GAACAAAGAACATTTTCACCAGTTC	300	521	Naderi, *et al.*, (2016)
	R	CTTTCAGGTGGGGAACCCG	320		

PCR Technique

The mixture of PCR Technique for amplification of all genes was explained in table 2. All PCR tubes were centrifuged for 1 min at a speed of 10000 rpm then transferred to the thermo cycler .Gradient PCR technique was carried out to determine the optimum annealing temperature. The amplification steps were programmed as shown in the table 2.

Table 2: The Condition of Amplification Process

Gene	Initial Denaturation (°C/min)	Number of cycle	Condition of Cycle			Final extension (°C/min)
			Denaturation (°C/sec)	Annealing (°C/sec)	Extension (°C/sec)	
lifA	94/5	35	94/30	58/30	5/72	40/72
Fimh	94/5		94/30	60/30		

Results and Discussion

Detection of *lifA* genes

The results of a garose gel electrophoresis of amplicon resulted from amplification of *lifA* in EPEC showed that only 19 (63%) isolates were possess this gene by appearance of amplicon with molecular weight 521 bp (Figure 1)

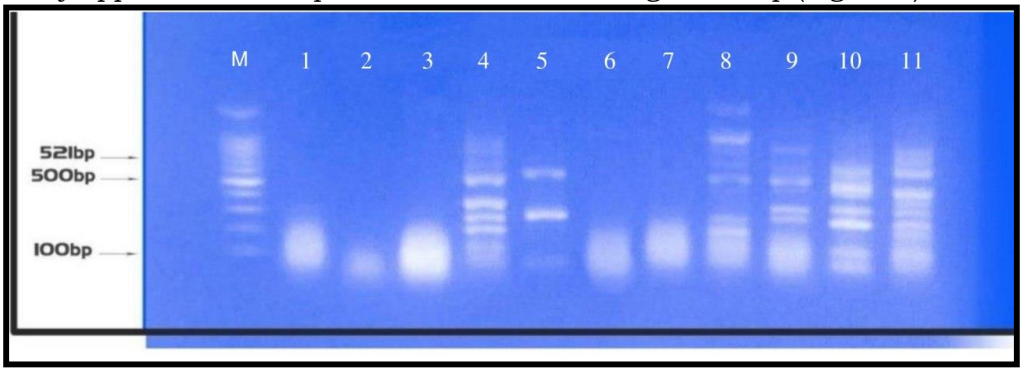

Figure 1: Agarose gel electrophoresis (1% at 80 volt for 1 hrs) of amplicon resulted from amplification of *lifA* (521bp) of EPEC. M: molecular DNA ladder. Lanes (1-2-4-5-6-8-10-12-13-14-18) positive results for amplification. Lanes (3-7-9-11-15-16-19-24-27) Negative results for amplification

LifA is a well-known virulence factor that functions as both a toxin and an adhesin, although its secretion and function are unknown. LifA is the largest type III effector found in any pathogen, with a calculated molecular weight LifA and other type III secreted proteins have been proven to be translocate into host cell (Deng, *et a,l 2012*). Typical EPEC might possess potential virulence factors (e.g., *efa1/lifA),* which is outside the LEE. The *efa1/lifA* gene has the product lymphostatin /Efa1, which inhibits the production of lymphokines and mitogen-activated proliferation of peripheral blood lymphocytes and gastrointestinal lymphocytes (Xu *et al., 2017*). The distribution of *lifA* was study in many previous stedies. XuY *et al.*, (2017) observed that *efa1/ lifA* was the gene with the strongest statistical association with diarrhea and *efa1/ lifA,* found to be more prevalent in EPEC strains. while Sirous *et al.,(*2020) confirmed that *efa1/lifA* gene was not observed in any of the EPEC .Slinger *et al.,* (2017) showed 42% of EPEC isolates were carriers *IifA* gene. Also Mercado *et al.*, (2016) indicated that 30.5% of EPEC isolates were carriers *IifA* gene. Lima *et al.*, (2019) noted that 40% of EPEC isolates were carriers *IifA* gene. Makobe *et al.*, (2012) mentioned that 19.8% of EPEC isolates were possess *IifA* gene. Memariani *et al.*, (2019) reported that of 16.7% of EPEC isolates were carriers *IifA* gene. Morabito *et al.*, (2003) noted that efa/lifA gene is involved in the capability of EHEC to adhere to cells and in the repression of the host lymphocyte activation response of EPEC. Narimatsu *et al.*, (2010) referred that the *efa1/lifA* gene may be true diarrhea pathogens. Badea (2003) revealed that *efa1/lifA* gene is adherence factor and, may play a role in adherence of EPEC infections that could then lead to diarrhea.

Detection of *fimH* genes

The results of a garose gel electrophoresis of amplicon resulted from amplification of *fimH* in EPEC showed that only 25(83%) isolates were possess this gene by appearance of amplicon with molecular weight 508 bp (Figure 2).

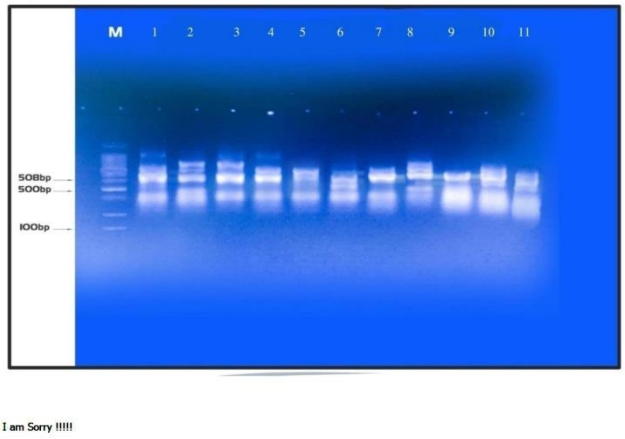

Figure 2: Agarose gel electrophoresis (1% at 80 volt for 1 hrs) of amplicon resulted from amplification of *fimH* (508bp) of EPEC. M: molecular DNA ladder. Lanes (1-2-3-4-5-6-7-8-10-12-13) positive results for amplification. Lanes (9-11-16-17-28) Negative results for amplification.

The high prevalence of *fimH* indicates a certain role for such gene in adhesion. *fim H* gene which codes for type 1 fimbriae, a critical for infection establishment(Cergole-Novella *et al.*,2015). The results of the present study reported that *fimH* gene was found in 25(83%) of EPEC isolates there was highly prevalence of *fimH* gene in all specimens . It is a function of Factor of colonization in extraintestinal infections and biofilm formation (Sarowska *et al.*, 2019). The distribution of *fimH* was studed in many previous studies. AL-Faham (2016) who the reported that 90% of EPEC isolates have *fimH* gene. Khairy *et al.*, (2016) pointed 91.4% of EPEC isolates were carries *fimH* gene. Abolmaali *et al.*, (2020) revealed that 95.33% of EPEC isolates were possess of the *fimH* gene. Ahmed (2016) showed that 90% of EPEC isolates have of the *fimH* gene. Also Abed (2013) referred that 100% of EPEC isolates were carries *fimH* gene. Abass (2014) found that 71% of EPEC isolates have *fimH*. Pourzare *et al.*, (2017) noted that 65.9% of EPEC isolates were carries *fimH* gene. Johnson *et al.*, (2019) mentioned that 85% of EPEC isolates were possess of the *fim H* gene. Tajbakhsh, et al., (2016) showed 85% of EPEC isolates have *fimH* gene. Aljanaby (2017) noted that 83.33% of EPEC isolates were carries *fimH* gene**.** Abd El-Baky, et al.,(2020)noted 78.3% of isolates were carries *fim H***.** while Ekundayo *et al.*, (2020) discovered that three E.coli bacterium strains (40/3) belonged to the Phylogenic group D type, were carriers of the *fim H***.** Tajbakhsh *et al.*,(2016) reported that 93.33% of EPEC isolates were possess *fim H*. Hojati *et al.*,(2015) conformed that 90% of EPEC isolates were carries *fimH*. Kargar, et al. (2014) and Yun, et al., (2015) indicated that *fimH* gene were most important virulence genes in E. coil strains isolated from fecal Samples.

FimH is one of the factors responsible for the ability of bacteria for adherence to the surfaces of epithelial cells and mucous membranes of the host cells and the necessary steps and basis in bacterial colonization, *fimH* encoding the adhesion protein in *E* coli (Foroogh *et al.*, 2021) which facilitate adhesion and colonization ofisolates to the target cells. The high binding ability of *fimH* could result in the increased pathogenicity of *E. coli*; thus *fimH* could be used as a possible diagnostic marker and/or vaccine candidate (Hojati *et al.*, 2015).

Conclusions

There was high incidence of *fimH* and *lifA* genes in EPEC isolated from children suffering from diarrhea.

References

Abass, S. K ; Ali, M. R ; & Authman, S. H. (2014). Isolation of Multi Antibiotics Resistance Escherichia coli from urinary tract infection and the Detection of PapC and fimH virulence genes by Polymerase chain reaction Technique. Diyala J Pure Sci, 10: 112-27**.**

Abd El-Baky, R. M ; Ibrahim, R. A ; Mohamed, D. S ; Ahmed, E. F ; & Hashem, Z. S. (2020). Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic E. coli isolated from Egyptian patients with different clinical infections. Infection and Drug Resistance, 13: 1221**.**

Abdalhamid, B ; Mccutchen, E. L ; Bouska, A. C ; Weiwei, Z ; Loeck, B ; Hinrichs, S. H ; & Iwen, P. C. (2019). Whole genome sequencing to characterize Shiga toxin-producing Escherichia coli O26 in a public health setting. Journal of Infection and Public Health, 12(6): 884-889.

Abed, S. N ; Kadhum, N. H ; & Abolmaali, H. M. (2020, December). Investigation of Escherichia coli FimH gene occurrence isolated from clinical and environmental samples. In AIP Conference Proceedings (Vol. 2290, No. 1, p. 020045). AIP Publishing LLC.

Aljanaby, A. A. J ; & Alfaham, Q. M. H. (2017). Research Article Phenotypic and Molecular Characterization of some Virulence Factors in Multidrug Resistance Escherichia coli Isolated from Different Clinical Infections in Iraq.

Badea, L ; Doughty, S ; Nicholls, L ; Sloan, J ; Robins-Browne, R. M ; & Hartland, E. L. (2003). Contribution of Efa1/LifA to the adherence of enteropathogenic Escherichia coli to epithelial cells. Microbial pathogenesis, 34(5): 205-215.

Bessaiah, H ; Anamalé, C ; Sung, J ; & Dozois, C. M. (2021). What flips the switch? Signals and stress regulating extraintestinal pathogenic Escherichia coli type 1 fimbriae (pili). Microorganisms, 10(1): 5.

Cergole-Novella, M. C ; Pignatari, A. C ; & Guth, B. E. (2015). Adhesion, biofilm and genotypic characteristics of antimicrobial resistant Escherichia coli isolates. Brazilian Journal of Microbiology, 46: 167-171.

Deng, W; Hong, B. Y ; de Hoog , C. L ; Stoynov, N ; Li, Y ; Foster, L. J ; & Finlay, B. B. (2012). Quantitative proteomic analysis of type III secretome of enteropathogenic Escherichia coli reveals an expanded effector repertoire for attaching/effacing bacterial pathogens. Molecular & Cellular Proteomics, 11(9): 692-709.

Foroogh, N ; Rezvan, M ; Ahmad, K ; & Mahmood, S. (2021). Structural and functional characterization of the FimH adhesin of uropathogenic Escherichia coli and its novel applications. Microbial pathogenesis, 161: 105288.

Hojati, Z ; Zamanzad, B ; Hashemzadeh, M ; Molaie, R ; & Gholipour, A. (2015). The FimH gene in uropathogenic Escherichia coli strains isolated from patients with urinary tract infection. Jundishapur Journal of Microbiology, 8(2).

Johnson, J. R., Johnston, B. D., Porter, S. B., Clabots, C., Bender, T. L., Thuras, P., ... & Banerjee, R. (2019). Rapid emergence, subsidence, and molecular detection of Escherichia coli sequence type 1193-fimH64, a new disseminated multidrug-resistant commensal and extraintestinal pathogen. Journal of clinical microbiology, 57(5), e01664-18.

Kargar, M ; & Homayoon, M. (2015). Prevalence of shiga toxins (stx1, stx2), eaeA and hly genes of Escherichia coli O157: H7 strains among children with acute gastroenteritis in southern of Iran. Asian Pacific journal of tropical medicine, 8(1): 24-28.

Khairy, R. M ; Mohamed, E. S ; Abdel Ghany, H. M ; & Abdelrahim, S. S. (2019). Phylogenic classification and virulence genes profiles of uropathogenic E. coli and diarrhegenic E. coli strains isolated from community acquired infections. PLoS One, 14(9):e0222441.

Lawal, O. U., Parreira, V. R., & Goodridge, L. (2022). The Biology and the Evolutionary Dynamics of Diarrheagenic Escherichia coli Pathotypes.

Lima, M. P ; Yamamoto, D ; Santos, A. C. D. M ; Ooka, T ; Hernandes, R. T ; Vieira, M. A., ... & Gomes, T. A. (2019). Phenotypic characterization and virulence-related properties of Escherichia albertii strains isolated from children with diarrhea in Brazil. Pathogens and disease, 77(2): ftz014.

Makobe, C. K ; Sang, W. K ; Kikuvi, G ; & Kariuki, S. (2012). Molecular characterization of virulence factors in diarrhoeagenic Escherichia coli isolates from children in Nairobi, Kenya. The Journal of Infection in Developing Countries, 6(08): 598-604.

McFadden J. F. (2000). Biochemical test for identification of medical Bacteria. 3rd ed. The Williams and Wilkins. Baltimore USA.; Pp.: 214-289.

Memariani, M ; Najar-Peerayeh, S; & Zahraei Salehi, T. (2019). Multi locus vntR (MLVA) typing and detection of the OI-122 pathogenicity island in typical and atypical enteropathogenic escherichia coli isolated from children with acute diarrhea. Arch Clin Infect Dis: 14: e65855.

Mercado, E. H ; Piscoche, C; Contreras, C; Durand, D; Riveros, M., Ruiz, J; & Ochoa, T. J. (2016). Pathogenicity Island O-122 in enteropathogenic Escherichia coli strains is associated with diarrhea severity in children from Lima Peru. International Journal of Medical Microbiology, 306(4): 231-236.

Morabito, S ; Tozzoli, R ; Oswald, E ; & Caprioli, A. (2003). A mosaic pathogenicity island made up of the locus of enterocyte effacement and a pathogenicity island of Escherichia coli O157: H7 is frequently present in attaching and effacing E. coli. Infection and Immunity, 71(6): 3343-3348.

Munhoz, D. D; Nara, J. M; Freitas, N. C ; Moraes, C. T ; Nunes, K. O ; Yamamoto, B. B; ... & Piazza, R. M. (2018). Distribution of major pilin subunit genes among atypical enteropathogenic Escherichia coli and influence of growth media on expression of the ecp operon. Frontiers in microbiology, 9: 942.

Narimatsu, H ; Ogata, K ; Makino, Y ; & Ito, K. (2010). Distribution of non-locus of enterocyte effacement pathogenic island-related genes in Escherichia coli carrying eae from patients with diarrhea and healthy individuals in Japan. Journal of clinical microbiology, 48(11): 4107-4114.

Nejma, I. B. S. B ; Zaafrane, M. H ; Hassine, F ; Sdiri-Loulizi, K ; Said, M. B ; Aouni, M., & Mzoughi, R. (2014). Etiology of acute diarrhea in tunisian children with emphasis on diarrheagenic Escherichia coli: prevalence and identification of E. coli virulence markers. Iranian journal of public health, 43(7): 947.

Pourzare, M ; Derakhshan, S ; & Roshani, D. (2017). Distribution of uropathogenic virulence genes in Escherichia coli isolated from children with urinary tract infection in Sanandaj, Iran. Arch Pediatr Infect Dis, 5(3): e41995.

Sarowska, J ; Futoma-Koloch, B ; Jama-Kmiecik, A ; Frej-Madrzak, M ; Ksiazczyk, M ; Bugla-Ploskonska, G ; & Choroszy-Krol, I. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut pathogens, 11(1): 1-16.

Sirous, M ; Hashemzadeh, M ; Keshtvarz, M ; Amin, M ; Shams, N ; Dastoorpoor, M ; ... & Koraei, D. (2020). Molecular Characterization and Antimicrobial Resistance of Enteropathogenic Escherichia coli in Children from Ahvaz, Iran. Jundishapur Journal of Microbiology, 13(7): 1-9.

Slinger, R ; Lau, K ; Slinger, M ; Moldovan, I ; & Chan, F. (2017). Higher atypical enteropathogenic Escherichia coli (a-EPEC) bacterial loads in children with diarrhea are associated with PCR detection of the EHEC factor for adherence 1/lymphocyte inhibitory factor A (efa1/lifa) gene. Annals of clinical microbiology and antimicrobials, 16(1): 1-6.

Tajbakhsh, E ; Ahmadi, P ; Abedpour-Dehkordi, E ; Arbab-Soleimani, N ; & Khamesipour, F. (2016). Biofilm formation, antimicrobial susceptibility,

serogroups and virulence genes of uropathogenic E. coli isolated from clinical samples in Iran. Antimicrobial Resistance & Infection Control, 5(1): 1-8.

Tareen, A. M ; Samad, A ; Mustafa, M. Z ; Maryam, M ; Rizwan, S ; & Akbar, A. (2022). Immunogenic protein profiling of pathogenic Escherichia coli strains isolated from infants with diarrhea in Quetta Balochistan. Journal of King Saud University-Science, 34(3): 101883.

Xu, Y ; Bai, X ; Jin, Y ; Hu, B ; Wang, H ; Sun, H ; ... & Xiong, Y. (2017). High prevalence of virulence genes in specific genotypes of atypical enteropathogenic Escherichia coli. Frontiers in cellular and infection microbiology, 7:109.