Original Article

Genotyping of Escherichia Coli Isolated from Diarrhea Cases in Children under Five Years of age Using the PFGE Method

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Abstract:

Twenty –five isolates of Escherichia coli isolated from cases of diarrhea in children under five years of age were obtained, characterized by Extensively Drug-Resistant(XDR) resistance patterns, and sensitivity screening of isolates was performed.E7 isolates were resistant to 4 groups of antibiotics and E5 - E13 - E14 - E17 - E18 - E20 - E47 - E46 - E42 - E31 - E26 isolates resistant to 5 groups of antibiotics and 48E isolate resistance to 6 groups of antibiotics and consisting of E2 E38 - E36 - E34 - E28 - E16 - E10isolates showed resistance to 7 groups of antibiotics, and E41 - E24 - E19 - E9 –E1 isolates resistance to 8 groups of antibiotics. The genotyping of 25 E.coliisolates was carried out by Pulsed-field Gel Electrophoresis (PFGE) using restriction endonucleases (Xbal) which belong to different phylogenetic groups, were as group A, group B2, group D (2/25 and 8%) group C (4/25 and 16%), group F (3/25 and 12%), group E (12/25 and 48%). The genetic convergence rate of isolates is 83%, and this is due to the similarity in genetic content, the source of isolation, and the place of isolation itself.

Keywords: Polycystic ovary syndrome, hepcidin, luteinizing hormone, prolactin.

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Introduction:

E.coli belong to Enterobacteriacea family, which is one of the pathological bacteria normally inhabitant in the human gastrointestinal tract and its possession of many factors of virulence, and its resistance to antibiotics has become an important pathogen[1], as it has the potential to opportunistically infect the human body whenever the necessary opportunity is available, causing several diseases [(1-3)].The factors causing diarrhea in infants and young children who are under the age of five are due to bacterial causes and viral, parasitic and fungal pathogens, most of which are transmitted through water contaminated with feces, the bacterial causes are: Enter pathogenic Escherichia coli, Shigella species, Salmonella spp., Vibrio spp., Clostridia spp. [4,5]The first strains of E.coli that colonized in the intestines of the newborn may have come from the mother's feces during childbirth or may have been through lactation.[5] Although the number of studies is small, Diarrheagenic E.coli (DEC) is the most pathogenic isolate, especially in association with the consumption of contaminated food and water, in low-income countries it is responsible for more than 35% of hospitalizations from these children under the age of five, Studies show that more than 3 million individuals will lose their lives by 2050 through MDR E. coli, which are spreading worldwide, MDR E.coli is now recognized as one of the greatest public health challenges worldwide (Magiorakos et al.,2020; Al-Kalifawi and Al-Azzawi ,2017).Treatment of E.coli infection is further complicated by the emergence of resistance to most first-line antimicrobial agents, cephalosporins resistance among members of Enterobacteriacea family

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has increased mainly due to the possession of β-lactamases enzymes and imipenem resistance.[7,8]

Pulsed-field Gel Electrophoresis (PFGE) is the technology that enables the separation of high molecular weight DNA molecules in agarose gel, as the DNA ranges from 10 KB to 800 KB by electrophoresis in agarose gel with an alternating electric field (pulsed) in two directions; this technology plays a key role in modern genomics because it allows the DNA separation of whole chromosomes or their large parts, it is a very distinctive technique with a wide capacity and high compatibility with epidemiological association, with the examination of a large part of the genome by (90%), the ability to differentiate between unrelated strains and also to prove the relationship between organisms derived from the same source and the classification of several bacterial species by choosing the enzyme and the optimal conditions for each species. [9]

The study aimed to conduct genotyping by the PFGE method to verify genetic convergence between E.coli strains of bacteria, and to prove the relationship between organisms isolated from the same source.

Materials and Methods:

Collecting Samples:

120 stool samples were collected from children with diarrhea for ages under five years and for both gender visiting hospitals, and under the specialized medical supervision in three hospitals (Children's Protection Teaching Hospital / City of Medicine, Central Children's Teaching Hospital and Al-Alwiya Teaching Hospital) in Baghdad for the period from January 2022 to the end of April 2022.

Isolation and Diagnosis of E.coli.:

E.coli samples were diagnosed using cultures media;MacConkey agar, Blood Agar and EosinMethylene Blue Agar, and biochemical assays; Catalase, Oxidase and IMViC which included, Indole Methyl red, Vogesproskauer and Citrate utilization (Tille, 2017). Final diagnosis of isolates using the Vitek-2 compact system(France, Biomerieux).

Sensitivity Test:

The sensitivity test for bacterial isolates under study was performed using the Kirby–Bauer method according to WHO (2003) for 15 antibiotics, which include:Amoxycillin (30µg), Ampicillin(10 µg), Cefepime(30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), NORfloxacin (10 µg) Chloramphenicol (30 µg), Amoxycillin/Clavulanic acid30µg), Aztreonam (30 µg), Piperacilli/ Tazobactam (100/10 µg), Trimethprim-Sulfamethozal (1.25/23.75 µg).

Genotyping using the (PFGE) Method for E.coli:

Genotyping of the 25 isolates was performed using the PFGE method as reported in (Pulse Net, 2017).

Running the Gel:

It is prepared by mixing 1.5 g of Sea Kem Gold agarose with 150 ml of TBE X (0.5) and dissolved in a microwave, then placed in a water bath at a temperature of 50-55 °C until use, after a period of incubation of the cutter enzymes wash the plugs, with 200 µl of TBE X0.5 for 5 minutes, then prepare the comb and put the sections of the enzyme-treated plugs and leave to dry in the air for 3 minutes, then the comb is clarified in place and the agarose is poured gently, and accurately in the jelly molding platform and leave to harden for 30-45 minutes.

Staining and Documentation:

The gel was removed from the room, and it was stained with an ethidium bromide solution (10 mg/ ml), by adding 50 µl of stock solution in 500 ml of D.W for 20 minutes in a covered container, then the gel was placed in D.W for (45) minutes and then the gel was examined using a UV-Trans-illuminator source at a wavelength of 260 nm, after which it was photographed with a high-resolution camera.

Standard Strain:

The standard isolation E.coli ATCC-25922 was used in this study and obtained from Central public health laboratory/ Iraq.

Interpretation of PFGE Patterns:

The genotyping obtained by the PFGE method were interpreted based on the Past program version 4.03

https://past.en.Io4d.com/windowsand then the phylogenetic tree was drawn to show the genetic convergence of E.coli isolates under study.

Results and Discussion:

Twenty –five isolates of diarrhea cases in children were obtained after laboratory tests and diagnosis of isolates, and when conducting an sensitivity test, the isolates were characterized by XDR resistance patterns,E7 isolate were resistant to 4 groups of antibiotics and isolates E5 - E13 - E14 - E17 - E18 - E20 - E7 - E47 - E46 - E42 - E31 - E26 resistant to 5 groups of antibiotics and 48E isolate resistance to 6 groups of antibiotics and isolates consisting of E2 E38 - E36 - E34 - E28 - E16 - E10. Genotyping was performed by the PFGE method to verify genetic convergence between E.coli strains.

The genotyping of 25 isolates was carried out by PFGE using restriction endonucleases (Xbal), which was characterized by resistance to 7 groups of antibiotics, and isolates E41 - E24 - E19 - E9 - E1 resistant to 8 groups of antibiotics, and the results of the study showed the presence of 6 patterns of genetic similarity for isolates of E.coli isolated from cases of diarrhea in children under five years, the molecular weight of these packages ranged between (50-1400) bp as shown in Figures (1) and (2).

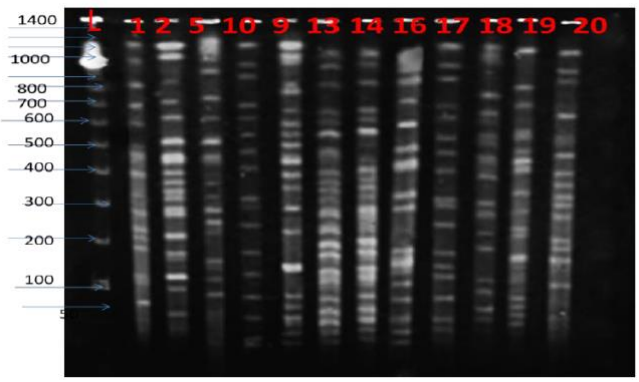

Figure (1): Pulsed-filed gel electrophoresis (PFGE) of E.coli isolates on agarose gel at a concentration of 1.5% field strength 6 V/cm, redirection angle 120°, for 21 hours. The L path represents the volumetric index (50-1000 kb) base pair for isolates of 1-2-5-10-9-13-14-16-17-18-19-20.

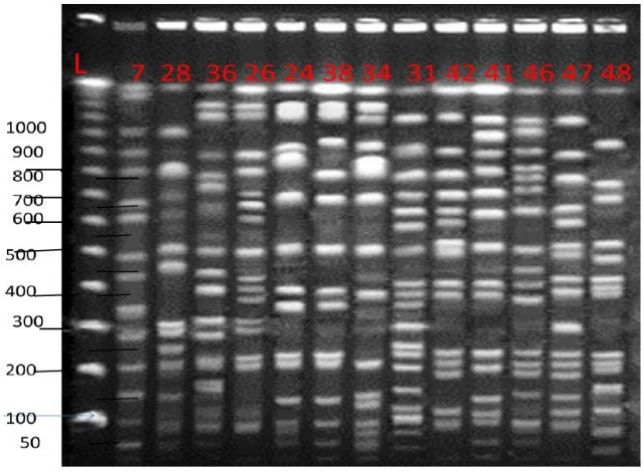

Figure (2): PFGE of E.coli isolates on agarose gel at a concentration of 1.5% field strength 6 V/cm, redirection angle 120°, for 21 hours. The L path represents the volumetric index (50-kb 1000 kb) base pair for isolates 7-28-36-26-24-38-34-31-41 - 42-46-47-48.

Genotyping was studied using the PFGE method, and 15 groups were identified as in Figure (3), as the results showed that the percentage of genetic convergence for the first type, which includes isolates (1-48), is 70%, and isolation (1) is due to the phylogenetic group (E), as the isolation (1) has genes (TrpBA, arpA, ArBAgpE, ChuA, TspE4c2, yjaA), and serotype (O111: K58). The isolation (48) belongs to the phylogenetic group (D), it has genes (TrpBA, arpA, ChuA, TspE4c2) and serotype (O142:K86), and that isolation (1) is resistant to (8) antigens and isolation (48) was resistant to (6) antigens, the second type, which includes each of the isolates (36,38,41,46,24, 34) and the genetic convergence rate of isolates (36.38) was 80%.

These two isolates are due to the same phylogenetic group (E) and serotype O55:K59 and resistance to 7 antigens, they are a cause of diseases resulting from contaminated food, food-producing animals such as cows and chickens are the main reservoirs of many foodborne pathogens, which are E.coli strains producing Shiga-toxins, direct contact with animals and consumption of dairy products or fresh fruits and vegetables [11-14]As for the isolates (41, 46), the genetic convergence rate of the isolates was 90%, the isolation (41) is due to the phylogenetic group (C), which has the genes (TrpBA, arpA, TrpAgp.C, TspE4c2), and serotype (O55:K59) and resistant to 8 antigens. The isolation (46) is due to the phylogenetic group (B2),

which has the genes chuA, yjaA, TspE4.C2 and the serotype O44:K59 and resistance to 5 antigens, and this convergence is due to the fact that the two isolates are isolated from the same hospital, which is the Children's Protection Teaching Hospital / City of Medical. The results showed convergence in genetic content also, the strains of the phylogenetic group (B2) are pathogenic and are associated with extra-intestinal infections and species of this group are usually isolated from carnivores and herbivores. The genetic convergence rate of isolates (36, 38) and (41, 46) was 85% because each of the isolates (36-38) and (41) was isolated from the same hospital. While the convergence rate between isolates (24-34) is 80%, as these two isolates have the same phylogenetic group (E) and have the same genetic content as they have genes (TrpBA, arpA, ArBAgpE, ChuA). Isolation (24) has serotype (O119:K6-), isolation (34) has serotype (O25:K11) and resistance to 7 groups of antibiotics, and the percentage of genetic convergence of the type that includes isolates (36,38,41,46) was 85%.The percentage of genetic convergence ratio of the type that includes isolates (36,38,41,46,24,34) was 83%, the third type includes isolates (19,28, 47,42) that the genetic convergence ratio of the two isolates (19,28) is 80%, the isolation (19) is due to the phylogenetic group (B2) and the serotype (O55:K59), which has genes (chuA and TspE4.C2). The isolation (28) is due to the phylogenetic group (E) that has the genes (chuA, TrpBA, arpA), and the serotype (O119: K69) and that the percentage of convergence is due to its isolation from the same hospital, which is the Central Children's Teaching Hospital, and both of these isolates are resistant to 7 and 8 antigens.The two isolates (19, 28) converge with isolation (47) by 90% and this isolation belongs to the phylogenetic group (E) that has genes (TrpBA, ArBAgpE, arpA, TspE4.C2),it included the serotype (O142:K86), and is resistant to 5 antibiotics, each isolates (19, 28, 47) are genetically close to the isolation (42) belonging to the same phylogenetic group (E) that has the genes TrpBA,ArBAgpE, TspE4.C2) and serotype (O44:K74), and resistance to 5 antibiotics. The genetic convergence rate of the type that includes the isolates (19, 28, 47, 42) is 95% and they were isolated from the same hospital, which is the Central Child Teaching Hospital.As for the fourth type, which includes isolates (9, 14, 31, 13, 18), the genetic convergence rate of the two isolates (9, 14) represents 90%, noting that the isolation (9), which is due to the phylogenetic group (E), which has the genes TrpBa, ArBAgpE, ChuA, TspE4c2, yjaA) and the serotype (O111:K58) and resistance to 8 antibiotics. Isolation (14), which belongs to the phylogenetic group (A), which has the genes (arpA, yjaA, TrpBA) and serotype (O44:K74), that E. coli isolates commensally are resistant to 5 groups of antibiotics(Clermont et al., 2015; Barzan et al., 2017b).The genetic convergence ratio between the two isolates [9, 14] with isolation (31) represents 95%, which belongs to the phylogenetic group (C) and serotype (O119:K69), as this phylogenetic group (C) was recently described (Moissenetet al., 2010), which has the genes TrpBA, arpA, TspE4c2, TrpAgp.C. Potential sources of DEC in this population may therefore be human or animal fecal matter. [15-22]

As for the genetic convergence between the two isolates (13 and 18), 80% were that the isolation (13), which is due to the phylogenetic group (A) and did not give a result for the serotype, this may be due to the presence of new patterns that have not been studied or because the methods of serotyping O- do not always indicate the exact pathogenicity of E. coli strains, and resistance to 5 antibiotics (Nakamura et al., 2021).Isolation (18), which is belong to the phylogenetic group (F), which owns the chuA gene and it included the serotype (O25:K11) and the percentage of genetic convergence between isolates (9,14,31) and isolates (13,18) by 88% and isolated from Al-Alawi Teaching Hospital. The fifth type, which includes each of the previously mentioned isolates (9,14,31,13,18) and isolates (2,7,20,5,26), includes the two isolates (2,7), the isolation (2) belongs to the phylogenetic group (F), which has chuA gene and belongs to the serotype (O142:K86).Isolation (7) belongs to the phylogenetic group (E), which has genes (TrpBA, arpA, ChuA), did not give a serotype, and the genetic convergence rate is 80%, both of which are transmitted through contaminated food, animals are the main reservoirs of many foodborne pathogens (Moxleyet al., 2020).The isolation (20) belongs to the phylogenetic group F, which has a gene (chuA), did not give a serotype, and resistance to 5 antibiotics, and the percentage of convergence is 88% between it and isolates (2, 7) and the convergence rate between the two isolates (5, 21) is 90%, as the isolation (5) belongs to the phylogenetic group (E), which included the serotype (O25:K11) and has the genes (TrpBA, arpA,

ChuA, TspE4c2, yjaA), while the isolation (21) belongs to the phylogenetic group (D), which has genes (TrpBA, arpA, TspE4c2, ChuA) included the serotype (O78:K80), and resistance to 5 antibiotics. The convergence rate is due to the fact that the potential sources of DEC in this population are human or animal fecal waste (Berthe et al., 2013) and to isolate them from the same hospital, the Central Children's Teaching Hospital, and they have the same genetic content. The sixth type includes each of the isolates (10, 17, 16), and the genetic convergence rate between the two isolates (10, 17) is 80%, and the isolation (10) is due to the phylogenetic group (E) and the serotype (O55:K59) and resistance to 7 antibiotics. The isolation (17) belongs to the phylogenetic group (C), which has the genes (TrpBA, arpA, TspE4c2, TrpAgp.C.), which included the serotype (O44:K74) and resistance to 7 antibiotics, and the percentage of genetic convergence with isolation (16) is 90%, which belongs to the phylogenic group (C) and the serotype (O78:K80) and resistance to 7 antibiotics, the genetic convergence is due to the fact that the two isolates were from the same phylogenetic group and has the same genetic content, the results of the study showed that the phylogenetic group (E) was the resistance to the largest number of groups, while the phylogenetic group (A) is resistant to the least number of antibiotic groups as shown in Table (1). Studies in different countries have reported a similarly high rate of resistance among E. coli isolates, this is related to the mechanism of antibiotic resistance produced by beta-lactamase enzymes , encoded by resistance genes and carried on plasmids, which can be exchanged between different bacterial species by horizontal transmission (HTG) and mobile genetic elements and resistance of Aminoglycoside (Mohammed, & Ibrahim, 2022; Mustafa and Abdullah, 2020).

The results of this research agreed with the findings of Lopes et al. (2022) in his study on 19 isolates of E.coli bacteria isolated from cases of diarrhea in children, in Mexico Cityand by PFGE method, which found that the genetic convergence rate of isolates is 73%, with 19 patterns and without subtypes, and it was found that they are multiple resistance to various antibiotics, which included fourteen antimicrobials: Ampicillin-sulbactam, Piperacillin-tazobactam, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime,Doripenem, eErtapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline

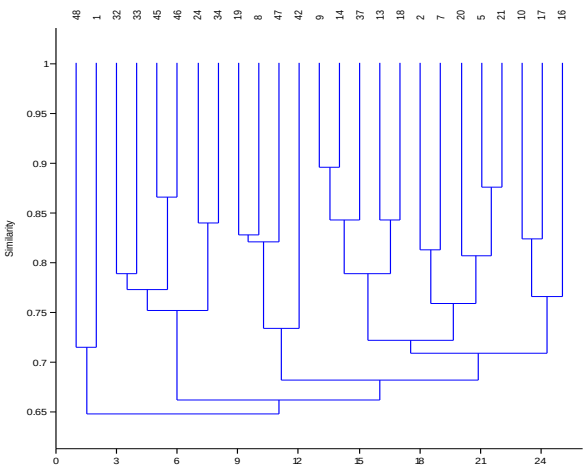

Figure (3): Phylogenetic tree of E.coli isolates using Past program version (4.03) https://past.en.Io4d.com/windowsby PFGE method for isolates (1,2, 5, 7, 9, 13, 10, 16, 17, 18, 19, 20, 14 21, 32, 24, 33, 37, 34, 42, 45, 46, 47, 48, 48).

Table (1): Percentage of Phylogenetic groups resistant to antibiotics of E.colibelongs to PFGE isolates.

Phylogenetic groups	Number of isolates	Isolates	Genes that owns	serotyping	Number of antibiotics that resisted	% of the isolates number
A	2	E14 13E	*arpAyjaA* *،TrpBA*	O44:K74 -	5 5	8
B2	2	E19 E 46	*chuA ،* *TspE4c2yjaA*	O55:K59 O44:K74	7 5	8
C	4	E16 E31 E41 E17	*TrpBAarpAyja* *ATrpAgp.C*	O78:K80 O119:K69 O55:K59 O44:K74	7 5 8 7	16
D	2	21E E48	*TrpBA , arpA* *chuA* *TspE4c2*	O78:K80 O142:K86	5 6	8

F	3	E2 18E E20	*chuA* *TspE4c2 ‹ChuA*	O142:K86 O25:K11 -	7 5 5	12
E	12	E7 E47 E5 E34 E42 E10 E36 E38 E28 E24 E1 E9	*TrpBAArBAgp* *EarpATspE4.C 2* *ChuAyjaA*	- O142:K86 O25:K11 O25:K11 O44:K74 O55:K59 O55:K59 O55:K59 O142:K86 O119:K69- O111:K58 O111:K58	4 5 5 7 5 7 7 7 5 7 8 8	48

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