ORIGINAL ARTICLE

The biosystematic of *E. coli* Strains Isolated from Clinical and Environmental Samples Phenotypically and Genotypically in Najaf Governorate

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ABSTRACT

Background: *The current work has employed the Clermont phylotyping approach to better elucidate the phylogenetic groupings of E. coli. A new technique called quadruplex PCR has been used to classify Escherichia coli (E. coli) strains into eight different phylogenetic groupings. However, it is still unknown how exactly these bacterial lineages relate to one another phylogenetically.* ***Objectives:*** *This study aimed to isolate, diagnose and classify E. coli bacteria into phylogroups and strains to determine the relationships between isolates taken from clinical and environmental samples in Najaf Governorate.* ***Methodology:*** *Samples collected from Najaf at a rate of 500 samples were subjected to culture, isolation and identification by biochemical tests and PCR technique based on the Clermont classification.* ***Results:*** *the positive samples were 250 isolates and seven phylogroups (A, B1, B2, C, D, E and F) were diagnosed in addition to the Unknown group and clade (I, II, V), as well as two pathotype groups (DEC/ EXPEC) and subpathotype groups affiliated with them (APEC, UPEC, SEPEC, MPEC). Also, commensal E. coli was identified. A completely new strain classification was established, and were recorded in the gene bank (NCBI), which classified under the term (Hetero-hybrid pathogenic), as plural of (EXPEC + DEC + DEC). Ten new E. coli isolates were registered in the (NCBI) as a first registration and an extension number was obtained.* ***Conclusion:*** *Using specific diagnostic primers, strains of E. coli bacteria are identified, and virulent strains recorded for the first time in Iraq were diagnosed and a new taxonomic branch designed for this study was adopted.*

INTRODUCTION

Worldwide, *Escherichia coli* is one of the most studied bacteria. These rod-shaped, Gram-negative, facultative anaerobic bacteria are found in the digestive tract where they typically colonize warm-blooded species, especially mammals but also birds, reptiles and fish. According to classification, *Escherichia coli* are a member of the Enterobacteriaceae family and an important member of the intestinal microbiota. Commensal relationships are those in which two species coexist without having obvious beneficial effects on each other, but also without causing harm to each other. In healthy humans, a variety of commensal microorganisms, primarily bacteria but also archaea and eukaryotes, inhabit the gastrointestinal tract[1]. The total number of cells in the microbial community, which includes more than 500 bacterial species, is ten times greater than the total number of cells in the body.

In the large intestine, the number of bacteria in the distal small intestine is higher and reaches 10^{11}-10^{12} colony-forming units per milliliter. The vast majority of bacteria in the large intestine are anaerobic organisms, with a 100:1 ratio of anaerobic to aerobic bacteria. Because of their metabolic adaptability, *E. coli* can also survive in environments other than those of their hosts, such as soil, water, plants, and food materials, although it forms symbiotic partnerships, this well-known microbe can also play a major detrimental role in its hosts, especially when it comes to human health[2].

The problem with lab techniques is unable to be used as certain bacteria cannot be cultured in the lab. they are used in conjunction with sequence analysis methods to overcome some of the difficulties of lab techniques. Most sequence analyses compare homologous genome segments to determine which organisms belong together. These methods are accurate but can be very time-consuming. Due to the time inefficiency and computer processing that these methods take, it is possible that new, heuristic approaches need to be considered. An alternative to comparing homologous segments is to compare the composition of genomes to discriminate between genomes of the same species [1,2].

METHODOLOGY

Identification of bacterial strains

A 250 *E coli* isolates were taken from specimens including, meat, fish, chicken, raw milk, cream, cheese, tap water, river water, stool and urine from November 2023 to June 2024 from general hospitals and Environmental of najaf Province, Iraq. All the isolates were cultured on blood and MacConkey agar (Merck, Germany). Colonies were identified by Gram stain and biochemical tests such as urea hydrolysis, H2S production,lysine decarboxylase, lactose ermentation, indole, methyl red, voges proskauer, citrate (IMViC) and oxidase tests[3]. In the current study, 250, 62(51.7%) specimenshave been appeared as positive result for *E coli* while 58(48.3%) of specimens were represented other Gram negative bacteria (Figure 2).

Virulence factors detection:

1. Biofilm : Biofilm production was assessed by detection of crystal violet retention after overnight broth growth in polystyrene microtiter plates described in Basnet, *et al.*[4].
2. Hemolysin production was determined on blood agar plates. It was achieved by Wang, *et a*l.[5].
3. Capsule: The negative staining bacteria of Indian ink was inquired to detect the presence of bacterial capsule, according to Atlas *et al*.[6].

Genotypical characterization

Molecular Investigation

Polymerase chain reaction (PCR) were using nine a specific primerin table 1. The amplification of genes was performed as follows: initial denaturation step at 94- 95˚C for 5min (one cycle), followed by 30 cycles consisting of denaturation at 94˚C for 30-60s, annealing at 50-63˚C for 30 to 60s and extension at 72C for 30s, and finalextension at 72 C for 10 min. The PCR products were visualized following electrophoresis on 1% agarose gels and staining with ethidium bromide.

The PCR data were then transmitted to the Macrogen firm in South Korea so that the AB DNA Sequence System. The quantity and kind of genetic mutations in the aforementioned gene were determined by reading the findings using the NCBI's (National Center for Biotechnology Information) BLAST (Basic Local Alignment Search Tool).

DNA sequencing:

Sequencing of PCR product DNA templates from reference strains *E. coli* were purified using QIA quick Gel Extraction kit (Qiagen, Germany), and sequenced by Bioservice Unit, Biotec, NSTDA, Thailand. The genomic DNA of the primers of the diagnostic gene 16SrRNA was analyzed after taking 20 micrograms/microliter of the amplified DNA of 10 of the studied isolates. After analyzing the results in the BLAST program available on the global website of the American gene bank National Center for Biotechnology Information (NCBI), it was found that the matching rate is very high with *E. coli*, so 10 isolates were registered in the NCBI as accession number (PQ326900, PQ327532, PQ326097, PQ327592, PQ327493, PQ326932, PQ325709, PQ326868, PQ327561, PQ326442).

Table 1: Specific primers used in this study

No.		Genes	Sequence 5-3	Size	References
1	Quadruplex	*chuA*	F 5-ATGGTACCGGACGAACCAAC-3 R 5-TGCCGCCAGTACCAAAGACA-3	288	Clermont *et al*., 2013
2		*yjaA*	F 5-CAAACGTGAAGTGTCAGGAG-3 R 5-AATGCGTTCCTCAACCTGTG-3	211	Clermont*et al*. (2013)
3		*TspE4C2*	F 5-CACTATTCGTAAGGTCATCC-3 R 5-AGTTTATCGCTGCGGGTCGC-3	152	Clermont *et al*. (2013)
4		*arpA*	F 5-AACGCTATTCGCCAGCTTGC-3 R 5-TCTCCCCATACCGTACGCTA-3	400	Clermont *et a*l., 2013
5	ArpAgpE.		F 5-ATTCCATCTTGTCAAAATATGCC-3 R 5-GAAAAGAAAAAGAATTCCCAAGAG-3	301	Lescat *et al*. (2012)
6	*f17A*		F 5-CAGAAAATTCAATTTATCCTTGG-3 R 5-TGATAAGCGATGGTGTAATTAAC-3	537	Bertin, *et al*., 1996
7	*hlyA*		F 5-AACAAGGATAAGCACTGTTCT GGCT-3 R 5-ACC ATATAAGCGGTCATTCCCGTCA-3	1177	Yamamoto, *et al*., 1995
8	*aggR*		F 5- CTA ATT GTA CAA TCG ATG TA-3 R 5- AGA GTC CAT CTC TTT GAT AAG-3	457	Czeczulin, *et al*., 1999
9	*16srRNA*		F 5'-GACCTCGGTTTAGTTCACAG-3' R 5'-CACACGCTGACGCTGACCA-3'	585	Wang et al., 1996

RESULTS

Phenotypical characterization

Biochemically:

Most of the *E coli* isolates were gave postive results for indol, MR and gas production and negative results for V.P simmon citrate. lipase, urease ,catalase and oxidase in addition to sugar fermentation as lactose .sucrose and maltose compared with the standard results in the table 2.

Table 2: Biochemical test and virulence factors of *E coli*

Types of Isolates 50 each sample	No. + / No. samples	Biochemical test									Virulence factors		
		Sorbitol fermentation	Catalase	Oxidase	Urease	Lipase	Indole	MR	VP	Simmon'scitrate	Hemolysis	Biofilm	Capsule
Milk	20	+	+	-	-	-	+	+	-	-	13/20 65%	13/20 65%	7/20 35%
Cream	16	+	+	-	-	-	+	+	-	-	9/16 56%	7/20 35%	6/16 37.5%
cheese	8	+	+	-	-	-	+	+	-	-	5/8 62.5%	3/8 37.5%	1/8 12.5%
River water	41	+	+	-	-	_	+	+	_	+1	38/41 92.6	28/41 68%	21/41 51%
Tap water	6	+	+	-	-	-	+	+	-	-	1/6 16.6	3/6 50%	0
Stool	39	+	-	-	-	-	+	+	_	-	29/39 74%	30/39 76.9%	18/39 46%
Urine	33	+1	-3	-	+1	-	-1	+	-	-	31/33 93.9	20/33 60.6	12/33 36%
meat	23	+1	+	-	-	_	+	+	-	-	10/23 43%	19/23 82.6%	8/23 34.7%
Fish	30	+2	-1	-	-	_	+	+	-3	-	25/30 83%	28/30 93%	12/30 40%
Chicken	34	+3	+	-	-	+1	+	+	-	+2	28/34 82%	26/34 76%	20/34 58.8%

Virulence factors:

The highest recorded hemolytic isolates were from urine samples, then river water (93.9, 92.6) respectively. As for the isolates producing biofilm, the highest recording of isolates within fish samples, then meat (93, 82.6), respectively. As for the capsule-producing isolates, the highest recorded isolates were in chicken samples, then feces (58.8, 46), respectively, as in table (2).

Molecular identification:

The result of detecting of *16S rRNA* gene in *E. coli* from (250) samples and specimens by PCR appeared that 100% bacteriall isolates were harbored the gene .

The study assessed the distribution of phylogroups in 250 isolates using the customized PCR method described by Clermont and associates, which the Table (3) shows the distribution of isolates according to Quadruplex patterns. The highest percentage was in category unknown group which is 74 (29.6%), Then comes category group E, clade I and B2 with a percentage of which is (26.4%, 20%) respectively.

The Quadruplex detection of phylogenetic group:

Conventional PCR was done in a Quadruplex patterns, were carried out to 250 *E. coli* isolates, the figures (The image is for illustration purposes only) of gel plates show the distribution of the four genes (*chuA*, *yjaA*, *TspE4C2*, *arpA*) across the samples and specimen , and Table (3) shows the distribution pattern to show the pathotype of *E. coli*.

Table 3: Distribution of isolates according to the phylo-group

No.	Quadruplex genotype				Phylo-group	Najaf n=250 (%)
	arpA	***chuA***	***yjaA***	***TspE4C2***		
1.	+	-	-	-	A	3 (1.2)
2.	+	-	-	+	B1	2 (0.8)
3.	-	+	+	-	B2	23 (9.2)
4.	-	+	+	+		21 (8.4)
5.	-	+	-	+		6 (2.4)
						50 (20)
6.	+	-	+	-	C, A	19 (7.6)
7.	+	+	-	-	D, E	10 (4)
8.	+	+	-	+		9 (3.6)
						19 (7.6)
9.	+	+	+	-	E, clade I	66 (26.4)
10.	-	-	+	-	clade II	10 (4)
11.	-	-	-	-	clade V	3 (1.2)
12.	-	+	-	-	F	4 (1.6)
13.	+	-	+	+	Unknown	17 (6.8)
14.	+	+	+	+		43 (17.2)
15.	-	-	+	+		8 (3.2)
16.	-	-	-	+		6 (2.4)
						74 (29.6)
17.	Total					250(100)

Molecular Detection of Virulence Genes:

Several virulence genes for *E. coli* isolates were examined using PCR and multiplex PCR, employing a primer unique to each gene and a thermocycler to verify the existence of the genes in concern in the isolates. All isolates were tested with (8) virulence genes (*hlyA, f17A, aggR , eae)* and Phylo-group (E,C) as in table (4).

Multiplex PCR was used to detect the genes of *aggR* measuring 457 bp, also multiplex PCR was used to detect the genes of *ArpAgpE* measuring 301 bp, while single-PCR was used to detect the genes of *eae* measuring 881 bp. Results showed the isolates had *aggR* gene 131 (19.5%).

Table 4: Distribution of isolates according to the virulence genes and Phylo-group (E,C)

Types of Isolates	No.+ / No. samples	Phylo-group (E,C)		Virulence Genes (%)			
		ArpAgpE	*trpAgpC*	*f17A*	*hlyA*	*aggR*	*eae*
Milk	20/50	20(100)	11(55)	10(50)	7(35)	8(40)	1(5)
Cream	16/50	14(87.5)	9(56.2)	11(68.7)	3(18.7)	10(62.5)	2(12.5)
Cheese	8/50	6(75)	1(12.5)	4(50)	3(37.5)	7(87.5)	0
River water	41/50	28(68.2)	20(48.7)	20(48.7)	17(41.4)	15(36.5)	1(2.4)
Tap water	6/50	6(100)	4(66.6)	4(66.6)	3(50)	4(66.6)	1(16.6)
Stool	39/50	37(94.8)	24(61.5)	21(53.8)	9(23)	18(46.1)	0
Urine	33/50	21(63.6)	10(30.3)	13(39.3)	12(36.3)	15(45.4)	1(3)
meat	23/50	22(95.6)	22(95.6)	16(69.5)	12(52.1)	15(65.2)	3(13)
Fish	30/50	19(63.3)	14(46.6)	15(50)	14(46.6)	19(63.3)	2(6.6)
Chicken	34/50	27(79.4)	18(52.9)	25(73.5)	18(52.9)	20(58.8)	2(5.8)
Total	250/500	200/250 (80)	132/250 (52.8)	139/250 (55.6)	98/250 (39.2)	131/250 (52.4)	13/250 (5.2)

Correlation between the isolates:

The distribution of all 250 samples on the cluster diagram Figure (1), the cluster groups were assigned numbers in order to calculate the percentage of similarity between the virulence factors and the biochemical test parameters.

The finding in cluster (1) (fish, chicken, one meat isolate, milk, cream, feces, urine, river water), as for cluster (2) it is a link cluster (1) and two separate milk isolates, while cluster (3) connects cluster (2) and a group that includes all types of isolates except tap water. Cluster (4) includes two groups, one of which contains (meat, milk, tap water, cheese, cream) and the other (meat, fish), in addition to two separate isolates of river water. Cluster (5) includes two groups (fish, milk), while the isolates included in cluster (6) were (chicken, stool , river water), as for the isolates included in cluster (7), they were represented by (stool, river water, cream, chicken), the group (river water, milk, chicken, urine, cheese, fish, tap water) belongs to cluster (8), as for clusters (3,4), they are connected by cluster (9), which in turn is connected to cluster (6) by cluster (11), cluster (13), which is connected by cluster (14) to cluster (10), which contains (Milk , cream).In cluster (15) includes (river water, urine, stool, fish) and is in turn linked to cluster (16). Which was linked by cluster (20) to the group (stool, fish), which was linked to the group containing (river water) under cluster (21).

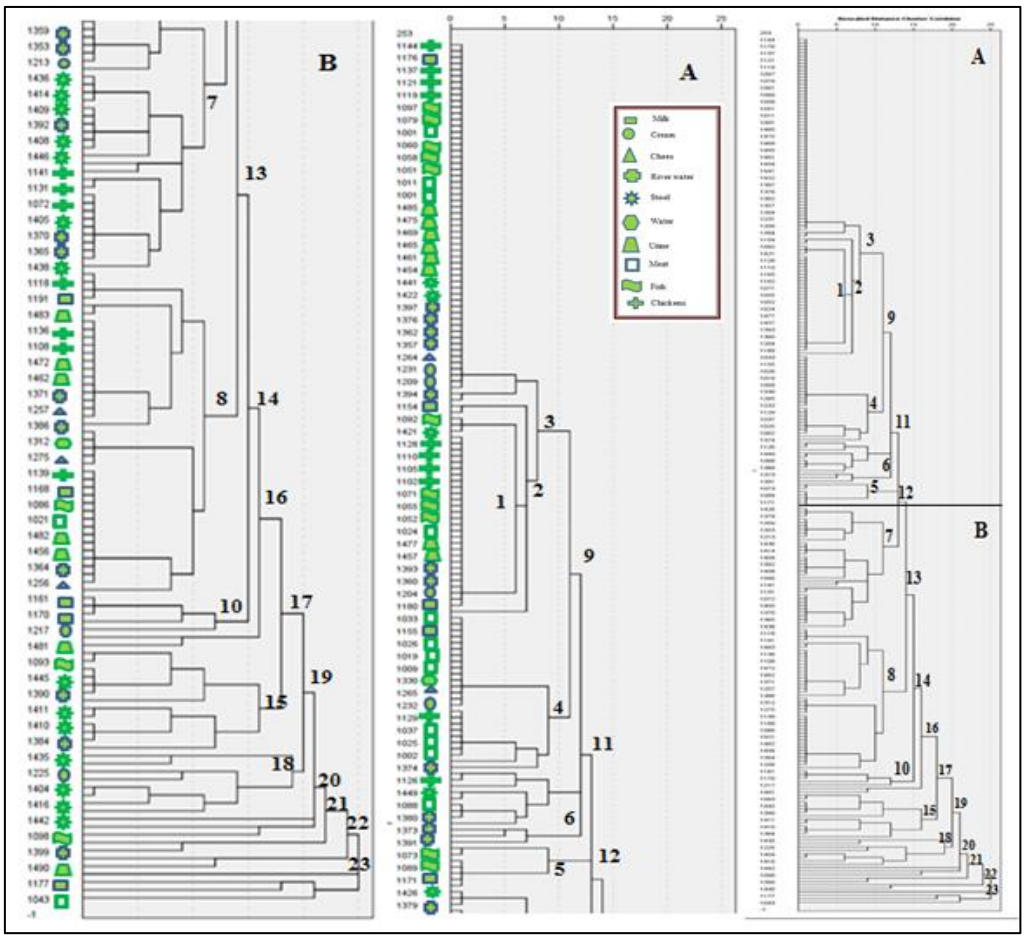

Fig. 1: Dendogram of correlation ship between of *E. coli* isolates in Najaf

Quadruplex PCR identification:

The figure (2) show the phylo-typing (A, Unknown, clade I) in meat samples, as were (1,3), (2,19,30/5,13, 18,20,24, 29) and (10,14,17, 21, 25, 28, 31) respectively , the result (B2=10%) , while the results of the figure (3) included isolates of meat distribution as clade I (32) , group E, clade I includes meat (33, 39, 43, 44, 45, 46,47, 52,54,55,56,57,58, 59), Unknown group includes (49,50,61,62/ 36,40,41,42, 48,60), group C,A (34), group F (51), This agrees with Clermont, *et al.*[7].

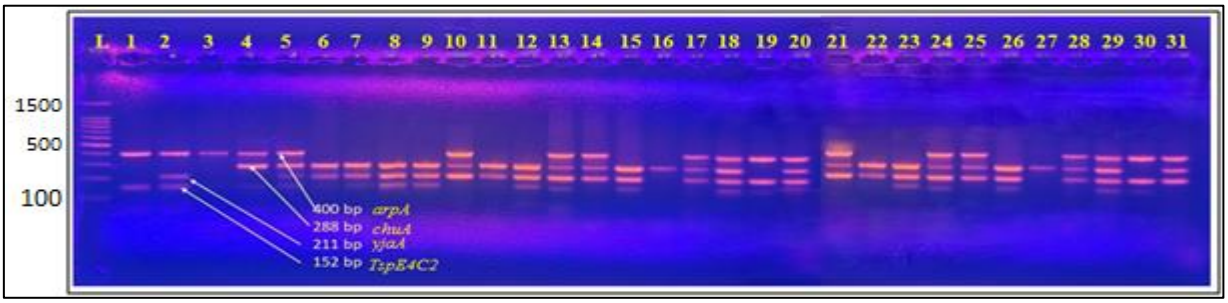

Fig. 2: The pattern Quadruplex PCR profiles of new Clermont phylo-typing method. Agarose gel electrophoresis was performed in 60 minutes at 70 volts, with the aim of targeting sequences such as arpA, chuA, yjaA, and TspE4C2. The PCR products were visible at 280 nm under UV light after being stained with ethidium bromide.

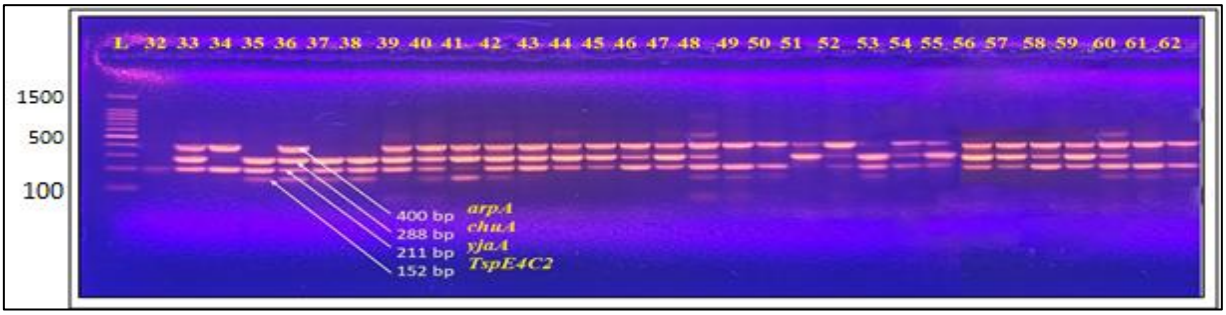

Fig. 3: The pattern Quadruplex PCR profiles phylo-typing . The objective of the 60-minute Agarose gel electrophoresis at 70 volts was to target sequences like TspE4C2, chuA, yjaA, and arpA. When the PCR products were stained with ethidium bromide, they were visible at 280 nm when exposed to UV light.

Virulence factors genes of *E coli* cobination

From the results we classify pathogenic group according to the combined virulence factors into:

1- **Hetero-pathogen strains**: - Carrying virulence genes shared by two or more DEC pathotypes[7]. The heteropathogens are therefore exclusively entero-pathogens, and their classification is predicated on the existence of certain DEC pathotypes linked to virulence factors. Since the genes that determine DEC are clearly specified, their definition is simple. This group appeared in our study as percentage of its isolates which as 75(30%).

2- **Hybrid-pathogen strains:-** Strains are either recovered from an extraintestinal infection and encode DEC defining virulence factors, or they show both DEC and ExPEC defining virulence factors[7]. The uncertainty in the gene sets needed to identify ExPEC strains necessitates the use of an alternative criteria for hybrid-pathogen naming. This group appeared in our study as percentage of its isolates was 12(4.8%).

3- **Hetero-hybrid pathogen strains:- (The term and its meaning are designed by this study),** The virulence factor genes from EXPEC are present in the strains in this category, which are hetero-pathogenes. These virulence factor combinations produce more severe diseases. Our analysis included this group, which as 36(15.6%) . The result Hetero-hybrid pathogenic is completely new and specific to our study and there are no similar results to compare between them.

***E. coli* Distribution according to the Pathogenic group:**

The tables (5) shows the types of *E. coli* groups in Najaf, as Pathogenic group consist of:

1. DEC (EHEC, EIEC, EAEC, EPEC, ETEC) in percentages (2.4, 2.4, 1, 1.2, 7.6) respectively, the highest frequency was (7, 6) within B2, Unknown respectively.
2. EXPEC (APEC, UPEC, MPEC) with percentage (6, 10.8, 3.2) respectively, the highest frequency was (11) within Unknown, followed by the frequency of Phylo-group E which is (8), then (6) within Phylo-group B2.
3. Commensal *E. coli*: It is appeared at a rate of 18(7.2%) distributed over five phylo-group (B2, C, D, E, Unknown) at a frequency was (2, 2, 1, 11, 2) respectively.
4. Hybrid pathogenic: Its subordinate isolates appeared at a rate of 73(29.2%) distributed between phylogroups (A, B2, C, D, E, F, Unknown) as (3,19, 5, 2, 21, 1, 22) respectively.

5. Heteropathogenic: Includes 16(6.4%) isolates appeared with phylogroups (A, B2, E, Unknown) as (1, 4, 1, 4, 6) respectively.
6. Hetero-hybrid pathogenic: The percentage of isolates belonging to it reached 38(15.2%), distributed as (B1, B2, C, E, F, Unknown) with frequency was (1, 8, 1, 16, 1, 11) respectively.
7. Cryptic Escherichia clades: Include (E. clade I, E. clade II) distributed as 9(3.6), 7(2.8) respectively.

Table 5: Types of *E. coli* according to the Pathogenic group

No.	Types of *E. coli* groups			phylo-group sensu stricto							Unknown	Total (%)
		Pathogenic group										
		Pathotype	Sub-pathotypes	A	B1	B2	C	D	E	F		
1.	A	1-DEC	EPEC			2	1	2	1			6(2.4)
2.			ETEC			3			3			6(2.4)
3.			EHEC			2			1			3(1.2)
4.			EIEC		1	1			2		1	5(2)
5.			EAEC			7	1		5		6	19 (7.6)
6.			DAEC									
7.			AIEC									
8.		2-EXPEC	APEC	2		3	1		1	1	7	15 (6)
9.			UPEC			6	1	1	8		11	27(10.8)
10.			NMEC									
11.			SEPEC									
12.			MPEC	1		2			3		2	8(3.2)
13.			ENPEC									
14.	B	Commensal				2	2	1	11		2	18(7.2)
15.	C	hybrid pathogenic		3		19	5	2	21	1	22	73(29.2)
16.	D	heteropathogenic		1		4	1		4		6	16(6.4)
17.	E	*Hetero-hybrid pathogenic			1	8	1		16	1	11	38(15.2)
18.	Total			7(2.8)	2(0.8)	59(23.6)	13(5.2)	6(2.4)	70(28)	3(1.2)	68(27.2)	
19.	cryptic Escherichia clades		E. clade I									9(3.6)
20.			E. clade II									7(2.8)
21.			E. clade V+									
22.	All samples and specimen											250(100)

DISCUSSION

E coli Isolateswere detected based oncultural, Morphological, microscopical and biochemical characteristics, according to MacFaddin[3]. Colonies of *E. coli* were observed, rounded, smooth,convex and Gamma hemolytic when cultured and streaked on BAB. While the colonies on MCA and EMB agar which appeared typically, mucoid, with pink to red pigment, usually diffusing into the surrounding agar, indicating fermentation of lactose and acid reduction. On the other hand, colonies found with yellow color on XLD agar as indicator for saccharide fermentation .The confirmatory cultured tests were performed by growing all 62 isolates of *E. coli* given greenish blue colonies on Chrome agar media[8,9].

All *E coli* isolates that grown on MCA, EMB agar, XLD agar undergo biochemical tests in order to distinguish *E. coli* isolates from other members of related lactose and sucrose fermented bacteria. All isolates of *E coli* were appeared lactose and sucrose fermenters. The biochemical tests that mentioned in Table (2) have been carried out for all *E coli* isolates under study according to MacFaddin[3]. the results were showed positive results for catalase test, while negative results for oxidase, lipase and protease test other isolates gave various results among clinical and environmental samples[10,11]. Lipase test results showed positive results for some samples, especially *E. coli* isolated from the cream, followed by samples isolated from tilapia fish, and river water, this is consistent with Machado *et al.*[12], Trang *et al.*[13]. The lipase and lipase-specific foldase genes were subcloned into two distinct expression vectors in order to boost the level of enzyme expression[14.15].

A *E coli* isolates under study were examined to inquire a capsule, using negative staining of Indian ink and examined to detected capsule phenotypeamong these isolates. The result of direct examination showing that 59(95.16%) of *E coli* isolates have capsule and

biofilm were 93% in addtion to hemolysis were 93.9. as in table (2). In summarized the relationship between many virulence factors like capsular, biofilm formation and hemolysis e play a major role in the severity level of *E. coli* Infections[16,17,18].

Genotypic characterazation:

The results of *16S rRNA* isolates from urine specimens agree with AL-Ammar and AL-Quraishi[9], while the diarrheal isolates consist with Tahir and Jabur[9]**.** As for the results of the remaining isolations from different sources, they agreed with Mounam, *et al.*[20]; Hong, *et al.*[21]. *16S rRNA*Gene appears in all isolates of the Enterobacteriaceae family, especially in the bacteria *E. fergusonii* and *E. albertii*, so it cannot be relied upon definitively to diagnose *E coli*, and this is consistent with Joshua, *et al.* [22]; AL-Huchaimi *et al.*[23].

Analysis of the destribution the samples according to the Quadruplex patterns:

The result of group (F) matched what was reached by Al-Khfaji *et al.*[18,] AL-Ammar and AL-Quraishi[19,] Mounam et al.[20], as it was (3.1%) is disagree with a recent study conducted in Baghdad, where their percentages for groups (A, B2, C, D, F) were (4%, 2%, 24%, 12%, 6%) respectively[24].

Pathotypes of *Ecoli* according to virulence factors genes:

Refer to strains that include virulence genes that are typical of two or more (DEC or EXPEC). The presence of certain virulence factors linked to these pathotypes determines the strain's classification. Since the genes that determine them (DEC or EXPEC) are well specified, their definition is simple.[21,22,23]

Ethical approval

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). Confidentiality of data, the authors declare that they have followed the protocols of their work center on the publication of patient data. Right to privacy and informed consent, the authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document. Use of artificial intelligence for generating text, the authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are accessible upon request.

Competing interests: There are no possible conflicts of interest that the author(s) of this article disclose with regard to the research, writing, or publishing of the piece. There is no current journal that is considering this submission, nor has it been published before.

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Conclusion: Using specific diagnostic primers, strains of E. *coli* bacteria are identified, and virulent strains recorded for the first time in Iraq were diagnosed and a new taxonomic branch designed for this study was adopted.

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