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The phylogenetic groupings of Escherichia coli isolated from human and farm animal feces in Basrah district, Iraq

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Abstract

The current work objective is to detect and differentiate Escherichia coli isolates from the feces of livestock animals and humans. Between September 2018 and January 2019, a total of two hundred and sixty-four fecal swabs and samples were gathered from different regions of Basrah. Eighty-five cow samples, ninety-four human samples, and eighty-five sheep samples were among the samples. The existence of E. coli was determined using conventional microbiological testing and molecular approaches (by PCR for amplification of the uidA gene). The findings of these procedures revealed that 50 (18.9%) of the examined samples had E. coli.

The E. coli isolates were then tested for phylogenetic groupings using quadruplex PCR employing four genes (chuA, yjaA, TspE4.C2, and arpA). In this investigation, seven phylogenetic groups were discovered, with group D having the largest proportion (44%) and group E having the lowest (20%). Other isolates belonged to groups F, A, B1, and C, with 8 percent, 6 percent, 4 percent, and 2 percent, respectively. However , 16% of isolates were unfit for other types (Unknown).

Keywords: *Escherichia coli*, *uidA,* Antimicrobial susceptibility, phylogenetic groups

Introduction

Escherichia coli bacterium is a Gram-negative, facultative anaerobe that does not sporulate and belongs to the Enterobacteriaceae family. Lactose fermentation (which creates acid and gas) is only found in this species, however it can ferment a wide spectrum of carbohydrates (**1**).

Escherichia genus includes five species*: albertii, coli, fergusonii, hermannii, and vulneris*. The Enterobacteriaceae family's type genus is Escherichia, and its type species is Escherichia coli. Although E. coli is found in the digestive system, especially in the large intestine, many strains are opportunistic or primary pathogens (**2**). Pathogenic E. coli can be classified as extra-intestinal pathogenic E. coli or intestinal pathogenic E. coli based on their virulence factors (**3**). Diarrheagenic *E. coli* is a major economic pathogen in newborn piglets, calves, and lambs. In pigs, post-weaning diarrheal illnesses are also severe. Extraintestinal infections may develop everywhere, including the urinary system, umbilicus, blood, lungs, and wounds, and they can affect any animal species. Septicemia is caused by *E. coli* in neonates of many species, but mainly in calves, piglets, lambs, foals, puppies, and kittens, as well as opportunistic septicemia in older immunosuppressed animals (**2**).

Clermont's phylogenetic categories were allocated to *Escherichia coli* strains based on the presence or lack of the genes chuA, yjaA, tspE4C2, and arpA. E. coli has eight recognized phylogroups, seven of which are *E. coli* (A, B1, B2, C, D, E, F) and one of which is Escherichia clade I (**4**). The population structure of *E. coli* can be used as a secondary bacterial source tracing method (**5**). Despite this, the phylogroup assignment given by (**6**), which categorized E. coli into the four major phylogroups A, B1, B2, and D based on the presence or lack of two genes (chuA and jayA) and a (TspE4.C2) fragment, is still valid. Clermont's initial technique was demonstrated to produce phylogroup assignments that were highly compatible with those acquired from MLST data (**7**). The purpose of this study was to isolate and characterize *E. coli* from

the stool and fecal swabs taken from people and domestic animals, as well as to molecularly detect phylogenetic groupings.

MATERIALS AND METHODS

Samples collection

The samples and swabs were gathered from several locations around the province of Basrah. From September 2018 to January 2019. From healthy cattle, sheep, and people, two hundred and sixty-four fecal samples and swabs respectively were collected, consisting of 85 bovine samples (80; 5), 94 human samples (10; 84), and 75 ovine samples (75; 10) .

Microbiological techniques

Bacterial isolation and characterization

The samples were handled according to the manufacturer's guidelines (**8**). In a nutshell, all of the samples were promptly delivered to the Veterinary College's laboratory through the cool box.

Samples were implanted in the nutrient broth while cultured overnight at 37°C in the laboratory. Mac Conkey's agar (Micromedia / Iran) was used to subculture the samples, which were incubated overnight at 37° C. The next day, 2-3 pink colonies were chosen at random and sub-cultured onto EMB agar (Himedia / India), then incubated overnight at 37° C. On the colonies with a metallic sheen, Gram's stain (**9**), indole test and methyl red (M.R), oxidase test and Voges – Proskauer tests were conducted according to (**10**).

Molecular techniques

Traditional microbiological procedures were used to verify *E. coli* isolates, which were then confirmed by PCR amplification of the *uidA* gene. A bacterial extraction kit was used to extract bacterial DNA according to the manufacturer's instructions (Genaid, Korea). The primer for the *uidA* gene (an E. coli housekeeping gene) was developed based on (12) the amplicon size (623bp) . Table (1).

Table (1): The primer sequences of *uidA* and their manufacturer

Primers name	Sequence of the Primers (5' to 3')	Primer Length	Size of the product	Reference	Manufacturers
uid A	F: 5'-CCAAAAGCCAGACAGAGT-3'	18	623 bps	12	Bioneer company / Korea
	R: 5'-GCACAGCACATCAAAGAG - 3'	18			

A total of 50 µl of PCR reaction mixture was prepared for the uidA gene. Ten µl of DNA template, 25 µl of master mix (USA), 2 µl of each primer, and 11 µl of Nuclease free water. For the *uid A* gene, the PCR conditions were as follows: denaturation at 94°C for 3 minutes, followed by (denaturation at 94°C for 1 minute, annealing at 56°C for 40 seconds, and extension at 72°C for 1 minute) for 30 cycles, with the final extension step at 72°C for 3 minutes.The conditions were adopted from (**11**).

Molecular detection of phylogenetic groups

Oligonucleotide primers for PCR amplification

Table (2) shows the primer sequences utilized in the identification of phylogenetic groupings, which were adapted from (**12**).

Table (2): Sequences of primers for phylogenetic group

Primers names	Sequence of the Primers (5′ to 3′)	Primer Length	Size of Products	Reference	Manufacturers
chuA	F 5′-ATGGTACCGGACGAACCAAC-3′	20	288 bps	13	ALPHA DNA
	R 5′-TGCCGCCAGTACCAAAGACA-3′	20			
yjaA	F 5′-CAAACGTGAAGTGTCAGGAG -3′	20	211 bps		
	R 5′-AATGCGTTCCTCAACCTGTG -3′	20			
TspE4.C2	F 5′-CACTATTCGTAAGGTCATCC -3′	20	152 bps		
	R 5′-AGTTTATCGCTGCGGGTCGC-3′	20			

arpA	F 5'-AACGCTATTCGCCAGCTTGC-3'	20	400 bps		
	R 5'-TCTCCCCATACCGTACGCTA-3'	20			

A total of 50 µl of PCR reaction mixer was prepared for genes. 10 µl of DNA template, 25 µl of PCR master mix (USA), one µl of each oligonucleotide primer, and 7 µl of Nuclease-free water. The conditions of amplification shown in table (3).

Table (3): The optimal amplification conditions for E. coli phylogenetic grouping

Stage	Step	Temperature	Time	No. of cycle
I	**Initial denaturation**	**94°C**	**4 min.**	**1**
II	**Denaturation**	**94°C**	**5 sec.**	**30**
	Annealing	**59°C**	**20 sec.**	
	Extension	**72°C**	**1 min .**	
III	**Final extension**	**72°C**	**3 min.**	**1**

RESULTS

Two hundred and sixty-four samples were obtained, composed of stool samples and swabs from livestock animals and humans. Standard microbiological and molecular methods were used to determine the E. coli isolation rate, as shown in table (4). Figure (1) shows how the isolates were identified as E. coli using the PCR technique to detect the uid A gene, which has a product size of 623 bps. Using standard biochemical analyses, 53 (20%) of the 264 samples were identified; nevertheless, 50 (94%) of the isolates were confirmed as E. coli.

Table (4) :Traditional microbiological methods and molecular approaches were used to identify the number of E. coli isolates.

Source of samples	Number of isolates identified using standard microbiological methods		Confirmed isolates by Molecular identification of the uidA gene.		Total number
	No.	%	No.	%	
Bovine	17	20	17	20	85
Human	10	10.6	9	9.6	94
Ovine	26	30.6	24	28.2	85
Total	53	20	50	18.9	264

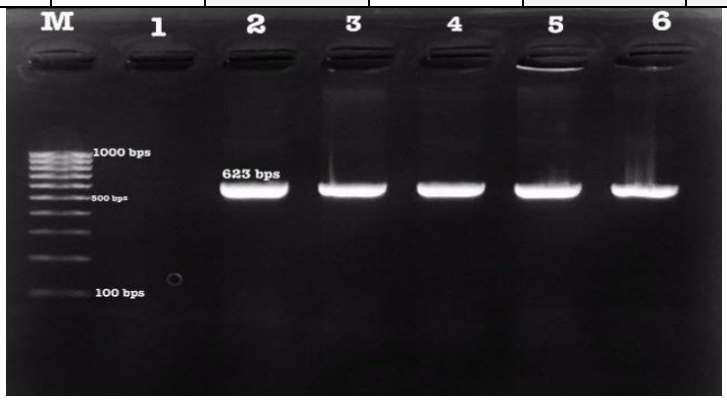

Figure (1): Electrophoresis of uidA gene amplification.

A 1.5 percent agarose gel was used to run the mixture. Ethidium bromide was used to stain it. M: marker, and the size of the uidA product was (623 bp). Well number (1) represents the negative control, , whereas the samples in wells 2–6 were positive.

Molecular detection of phylogenetic groups

Table (5) and figure (2) show how *Escherichia coli* isolates were categorized using PCR and amplification of genes such as (chuA, yjaA, TspE4.C2, and arpA).

Table (5): Phylogenetic groupings of E. coli distributed based on animal species.

Isolat	A		B1		C		D		E		F		UN	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%

estype														
Cattle	1	5.9	0	0	0	0	6	35.3	1	5.9	4	23.5	5	29.4
Human	2	22.2	1	11.1	0	0	5	55.6	1	11.1	0	0	0	0
Sheep	0	0	1	4.2	1	4.2	11	45.8	8	33.3	0	0	3	12.5
Total	3	6	2	4	1	2	22	44	10	20	4	8	8	16

Note: The percentages in row cells are calculated by dividing the number of isolates by the total number of isolates in the row. There were no isolates found in group (B2).

In this investigation, seven phylogenetic groupings were discovered. 22 (44%) of the 50 isolates belonged to group D, while 10/50 (20%) belonged to group E. Other isolates, however, belonged to groups F, A, B1, and C, accounting for 8%, 6%, 4%, and 2% of the total. On the other hand, 16% of isolates were unsuitable for other groups (Unknown). Except for groups B and C, the difference between groups was statistically significant ($P > 0.05$).

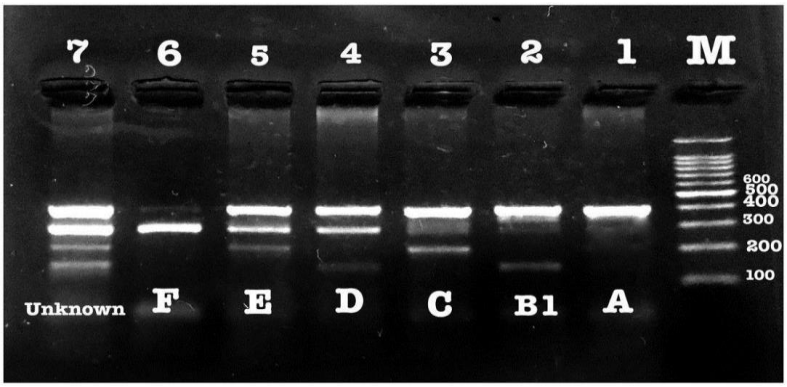

Figure (2): Amplification patterns of different phylogenetic groups.

A 1.5 percent agarose gel was used to run the mixture. Ethidium bromide was used to stain it. M: Markers. Phylogenetic groups of different types (1:A, 2: B1, 3:C, 4: D, 5:E, 6:F and 7: Unknown).

Discussion

***E. coli* isolation rates**

Conventional microbiological approach to identifying E. coli relies on enriching the sample in nutrient broth, then recognizing it on MacConkey agar, cultivating on EMB agar, and subjecting it to confirmation by biochemical and molecular approaches, as maintained by (**8**).

From the total number of samples, the E. coli was isolated from (18.9 %) of samples. E. coli was identified in 9.6% of the human samples. This result is lesser than the one reported in (**13**), which revealed the presence of E. coli in fecal culture (21.4 %).The isolation rate of *E. coli* in cattle in this study is (20%), which is more than (10.9%) that published by (**14**) in the Basrah district. In contrast, the average *E. coli* isolated from sheep was (28.2%).

Molecular detection of phylogenetic group

The phylogenetic characterization is required to better understand E. coli populations and the connection between strains and disease (**15**). The growing body of multi-locus sequence analysis and genomic data of E. coli has led to the identification of 8 E. coli phylogroups: (A, B1, B2, C, D, E, F) are Escherichia coli sensu stricto, while the last is E. coli cryptic clade I. A quadruplex genotype matching to the existence of the 4 genes is discovered for each strain [arpA, chuA, yjaA, and TspE4.C2] (**12**).

Seven phylogenetic groups were identified based on the prevalence and distribution of phylogenetic groups in 50 *E. coli* isolates from cattle, humans, and sheep. The isolates in this investigation were mostly from phylogenetic groups D and E (44 % and 20%, respectively). This result concurred with (**16**), who stated that the majority of the isolates belonged to phylogenetic groups D, as well as (**17**), who stated that the E group were identified in (20 %) of isolates. The findings of this study, on the other hand, contradicted those of (**18**), who discovered that the main phylogenetic group was B2. Group C was a low-detected group in the current investigation (2 %).

Only 23.5 percent of cow isolates belonged to group F; these isolates have a special relevance since they belong to extra-intestinal diseases; this result was higher than that of (**19**) who discovered this phylogroup only in 1.75 percent of cattle isolates.

Some E. coli strains found in this study were unsuitable for other groups, thus they were classed as unknown groups (16 %). Clermont et al., (2013) found that some strains were unsuited for other groups due to a combination of the presence and lack of particular genes, which occasionally resulted in the emergence of unclassified phylogroups (Unknown). Also (**12**), explained that because of the phylogroup's rarity, certain E. coli strains were unable to be assigned to any known phylogroups.

In conclusion, this study identified seven phylogenetic groups, with group D having the greatest ratio and group C having the lowest.

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