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Molecular Characterization Phylogenetic Analysis from *E.coli* Isolated from Human and Cows Raw Milk

Hasanain Jameel Ali[1], Zahraa Hussein Abdulateef Ali[2]

[1*] Department of Health Management Techniques, Najaf Technical Institute, Al- Furat Al-Awsat Technical University, Iraq.

[2] Department of Preventive and Orthodontic Dentistry, Najaf Technical Institute, Al-Frurat Al-Awsat Technical University, Iraq

SUMMATY

E. coli is a significant pathogen that contributes to the genesis of both human and animal diseases. 20 different *E. coli* isolates were found in milk, stool, and utensil samples. Depending on whether each *E. coli* strain has the gene, the chuA, and the DNA segment TspE4.C2 has joined. the intestinal groups A and B1 as well as the extraintestinal groups B2 and D. The findings of the PCR-based analysis revealed that the B2 $_3$ subgroup contained 6 feces samples, 4 milk samples and 2 utensil samples, where these groups are separated into six subgroups (A_0, A_1, $B2_2$, $B2_3$, D1, D2). While one stool sample, three milk samples, and one utensil sample were D2 subgroup members, two stool samples and one milk sample were A_1 group members (intestinal)., the results pointed to However, according to our data, most of the isolates are part of the phylogenetic B2 and D (Extra-intestinal) groups. Because the B2 and D groupings are very common, there could be a potential risk of exposure to pathogenic *E. coli.*, In this investigation, *E. coli* isolated was divided into phylogenetic groups according to the existence or absence of three genes: *chuA* , *yjaA* , and a DNA fragment termed *TspE4.C2* .,The B2 $_3$ group 3, in instance, was where a significant proportion of the detected isolates, stated that the presence of the B2 3 subgroup in faeces, milk, and utensils could be an indication of contamination

KEY WORDS: Phylogenetic analysis*, E. coli,* Cows, Raw milk, Human

INTRODUCTION

Escherichia coli lives normally in the gut as an intestinal commensal. Many species including humans. Numerous gastrointestinal and extra-gastrointestinal illnesses, including infections of the urinary tract, diarrhea, septicemia and newborn meningitis, can be caused by some strain of *E.coli* (1). According to phylogenetic study*, E. coli* strains can be split into four separate phylogenetic groups (A, B1, B2, and D). Group B2 and to a lesser extent, group D, constitute many harmful extra-intestinal strains (2,3).

Most cases, either observational comparisons of carefully defined populations or individual experimental studies are used to evaluate specific bacterial characteristics as markers for or factors to extraintestinal pathogenicity types of *E. coli* (4). Our prior analysis of isolates from acute *E. coli* infections from same-host milk and feces revealed that compared with isolated fecal strains, the strained milk is usually both more plentiful and highly pathogenic (5). *ExPEC* & DEC are 2 separate *E. coli* groups that could also, respectively, induce urinary tract infections or enteric disorders.

According to certain studies, food contamination by *E. coli* is a product of consuming food (6-7). Researchers have suggested examining antibiotic resistance in bacteria like *E. coli..* It would be very useful to use samples from healthy animals because species may be used to monitor the prevalence of antibiotic resistanceThese organisms may store the genes of resistant bacteria that are transferred from pathogenic bacteria to people either from humans to animals directly or through the food supply chain. It has been shown that resistant animal germs can make people sick (8-9).

MATERIALS AND PROCEDURE

Collection of samples and Method

A total of 190 samples were collected of various sites (60 cows milk, 60 stool human and 70 utensils samples). The obtained samples, which have been transported to the lab in a cold box, were analyzed within 24 hours. During the period from November 2024 to February 2025.

On MacConkey agar, all *E. coli* isolates make it suitable, and colonies usually have a large, Lactose fermentation is indicated by a pink form with pink pigment that usually diffuses into the surrounding agar. E. coli colonies on EMB agar have a distinct green sheen metallic, though. The identification of isolates was established using the API-20 E kit (bioMérieux - France). The extraction of DNA using a method that included employing (Gene aid extraction Kit). The nanodrop system was used to estimate the DNA concentration (10)

Specific PCR-amplified genes, including such chuA , yjaA , and TspE4, were expressed. Researchers were able to recognize the phylogenetic groups of E. coli are isolated from different substrates due to C2. Table (1). figure (1,2,3)

For the various pathogenic gene coding sites, specific PCR testing primer sequences and predicted amplification product sizes were used.

Table (1) The chuA, yjaA, and TspE4.C2 genes were amplified in a reaction volume of 20 µll to use an amplification technique that includes 3 stages of denaturation at 95 C, 30 cycles of annealing at 60 C, extension at 72 C, and a final extension at 72 C for 10 Min.

Table1: PCR amplification sequences and anticipated lengths, as well as the end products of the oligonucleotide primers utilized

Primer	Primer sequence (5-3)	Product	Source
chuA	F- GACGAACCAACGGTCAGGAT R- TGCCGCCAGTACCAAAGACA	279 bp	11
yjaA	F-TGAAGTGTCAGGAGACGCTG R-ATGGAGAATGCGTTCCTCAAC	211 bp	11
TspE4.c2	F-GAGTAATGTCGGGGCATTCA R-CGCGCCAACAAAGTATTACG	152bp	11

Results

The phylogenetic groups of E. coli w determined thanks to the presence of specific PCR amplified genes, including *chuA* , *yjaA* , and *TspE4C2*. several sources of E. coli Table (2).

Table (2) : Phylogenetic categories of *E. coli* isolated for milk, stool , utensils samples according to PCR results.

Isolates NO.	Virulence genes			Phylogenetic Group	Phylogenetic Subgroup
	chuA	*yjaA*	*TspE4.C2*		
1S	+	+	+	B2	$B2_3$
2S	+	+	+	B2	$B\ 2_3$
3S	+	+	+	B2	$B2_3$
4S	+	+	+	B2	$B2_3$
5S	+	+	+	B2	$B2_3$
6S	+	-	+	D	D2
7S	+	+	+	B2	$B2_3$
8S	-	+	-	A	A_1
9S	-	+	-	A	A_1
10M	+	-	+	D	D2
11M	+	-	+	D	D2
12M	+	-	+	D	D2
13M	+	+	+	B2	$B2_3$
14M	+	+	+	B2	$B2_3$
15M	+	+	+	B2	$B2_3$
16M	-	+	-	A	A_1
17M	+	+	+	B2	$B2_3$
18U	+	+	+	B2	$B2_3$
19U	+	+	+	B2	$B2_3$

20U	**+**	**-**	**+**	**D**	**D2**

Table 3: Distribution of phylogenetic groups *E.coli* isolates

Phylogenetic group NO. (%)		**Phylogenetic sub groups**	**No. (%)**	**Total NO. (%)**
Intestinal Group	**Group A**	**Subgroup A_0** ***chuA - / yjaA - / TspE4.C2-***	**0 (0)**	**3 (15)**
		Subgroup A_1 ***chuA - / yjaA + / TspE4.C2-***	**3(15)**	
	Group B1	***chuA - / yjaA - / TspE4.C2+***	**0(0)**	
Extraintestinal Group	**Group B2**	**Subgroup $B2_2$** ***chuA + / yjaA + / TspE4.C2 -***	**0(0)**	**17 (85)**
		Subgroup $B2_3$ ***chuA +/ yjaA + / TspE4.C2+***	**12 (60)**	
	Group D	**Subgroup D1** ***chuA + / yjaA - / TspE4.C2 -***	**0(0)**	
		Subgroup D2 ***chuA + / yjaA - / TspE4.C2 +***	**5(25)**	
Total (%)			**20 (100)**	

The Phylogenetic group were determined by the PCR technique. According depending on whether the genes *chuA* , *yjaA* , and a DNA fragment *TspE4.C2* was present at the same time or not within each strain of E. coli, groups A and B1 (intestinal groups), B2 and D (extraintestinal groups), When these groups were divided into six subgroups (A_0, A_1, $B2_2$, $B2_3$, D1, D2), the PCR-based results showed that the $B2_3$ subgroup were composed of 6 stool samples, 4 milk samples, and 2 utensil samples. While one stool sample, three milk samples, and one utensil sample were D2 subgroup members, two stool samples and one milk sample were A1 subgroup members (intestinal). The data also showed that subgroup $B2_3$ (extraintestinal) contained 12 isolates, which represented for 60% of the entire, followed by subgroup D2 (55%), then subgroup A_1 (3 isolates), which composed 15% of the total , No of these isolates was found to belong to group A_0, B1and $B2_2$ table (3)

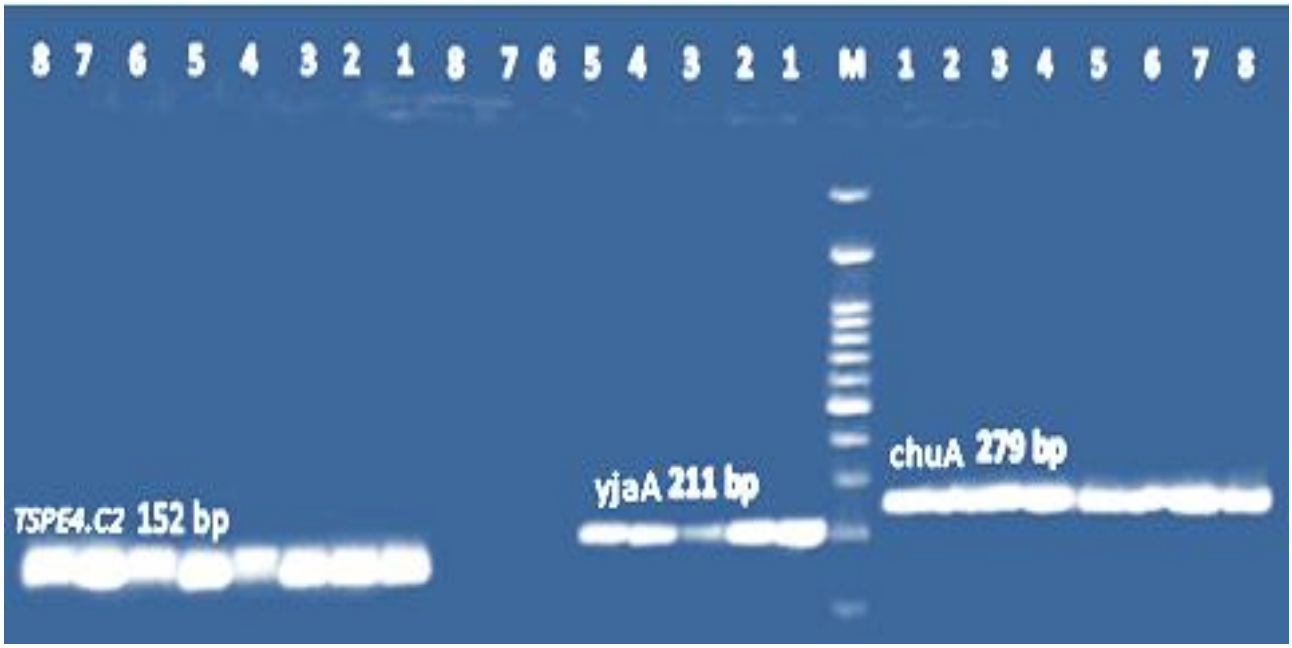

Figure 1: Agarose gel electrophoresis of *chuA* , *yjaA* genes and DNA segment *TSPE4.C2* at 82 volt for 30 min . M:3000bp ; Lane[1-6] stool samples (7-8) milk samples.

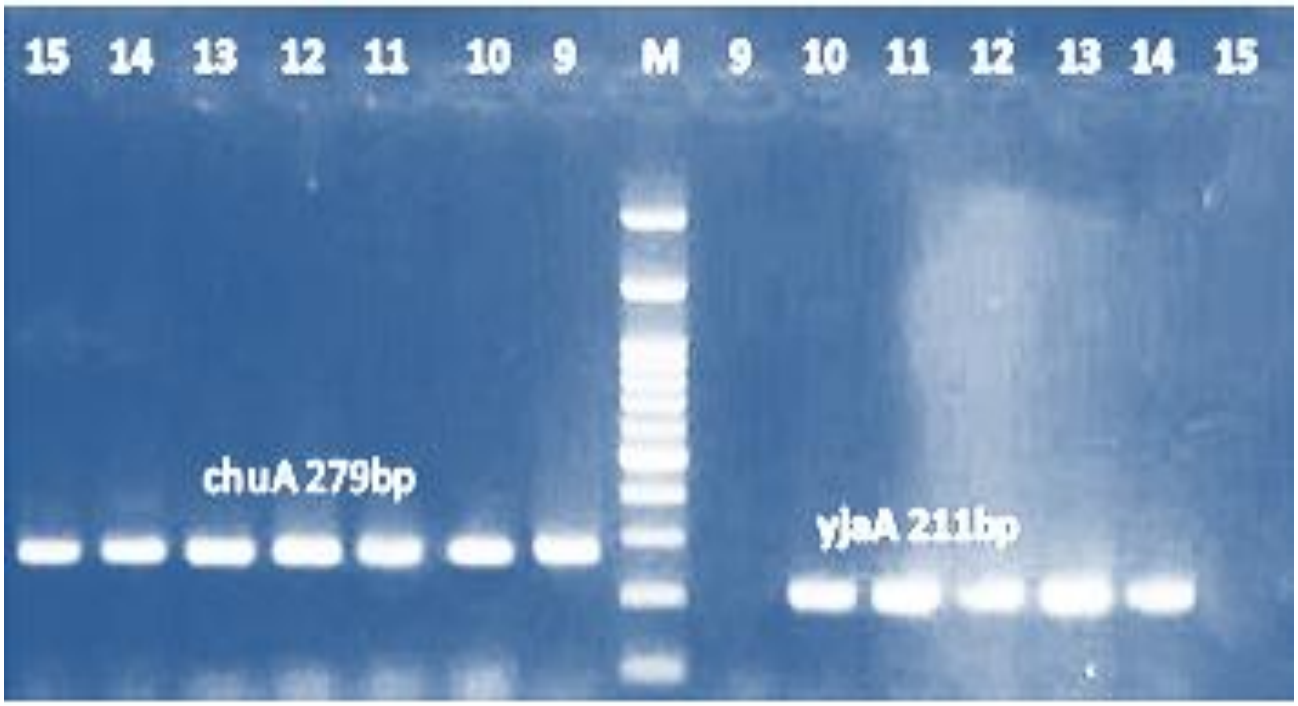

Figure 2 : Agarose gel electrophoresis for *chuA* and *yjaA* genes at 82 volt for 30 min. M:3000bp ; Lane[9-12] milk samples (13-15) utensils samples.

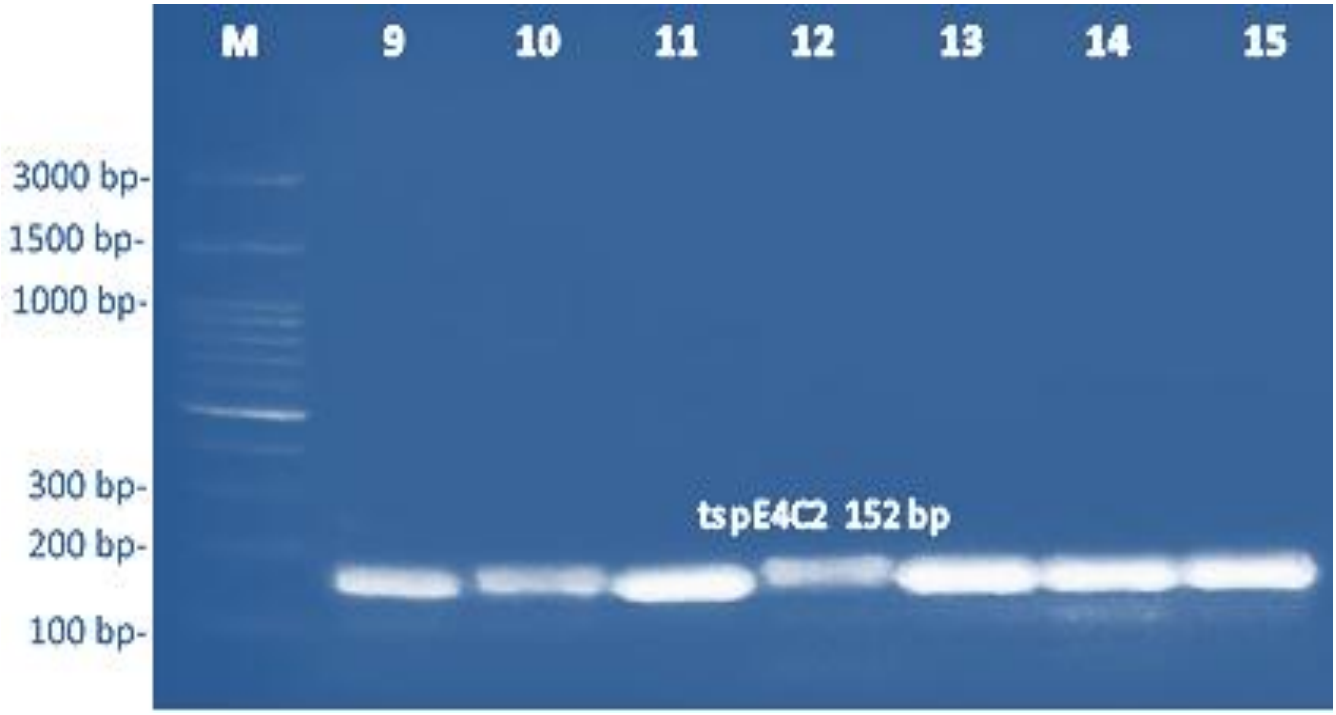

Figure 3 : Agarose gel electrophoresis for DNA fragment TspE4.C*2* at 82 volt for 30 min . M:3000bp ; Lanes [9-12] milk samples (13-15) utensils samples.

4. Discussion

E. coli strains can be divided into four distinct phylogenetic groups are (A , B1, B2- and D) (intestinal groups); A and B1 (extraintestinal groups). Group A is split into small groups in B2 and D Groups. A_0 and A_1 and group B2 groups $B2_2$ and $B2_3$ and group D to D1 and D2 (12).

In this investigation, *E. coli* isolated was divided into phylogenetic groups according to the existence or absence of three genes: *chuA , yjaA* , and a DNA fragment termed *TspE4.C2* (13).

The current study found all *E.coli* isolates was most prevalent in groups B2 and D while the least prevalent A group. the present results are in line with some studies published by (14) in Al-Hillah. who mentioned the group B2 contained the majority of isolates (75.47 %) , who reported higher percentage belonged to group B2 and D 56.7% and 20.7 % respectively(15).

The prevalence and dispersion of phylogenetic subgroups of *E. coli* isolates from milk, faeces, and utensils are displayed in this study were sub-A_1 , A_0 , B1, $B2_2$, $B2_3$, D1, D2. this result near from result reported by in Thi-Qar, Iraq (15) .

However, according to our data, the majority of the isolates are part of the phylogenetic B2 and D (Extra-intestinal) groups. Because the B2 and D groupings are very common, there could be a

potential risk of exposure to pathogenic *E. coli.* The B2 $_3$ group 3, in instance, was where a significant proportion of the detected isolates. stated that the presence of the B2 3 subgroup in faeces, milk, and utensils could be an indication of contamination (16).

5.Conclusion

Most E. coli isolates isolated from milk, stool, and utensil samples were found to be members of extraintestinal phylogenetic groups B2 and D, based to a PCR-based phylogenetic study that focused on the chuA, yjaA, and TspE4.C2 DNA fragment. However, the intestinal A1 subgroup made up just 15% of all isolates, whereas the B2-3 subgroup made up 60% of all isolates, followed by the D2 subgroup at 25%.

According to this distribution, extraintestinal pathogenic E. coli strains—which are well known for their capacity to cause septicemia, urinary tract infections, and other serious diseases in humans—may be significantly stored in raw milk and contaminated utensils.

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