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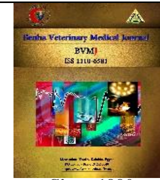

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Original Paper

Serotyping and phylogenetic grouping of pathogenic *Escherichia coli* strains isolated from milk and milk products.

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ABSTRACT

Escherichia coli represents as one of gut microflora of worm- blooded animals that distributed widely. This bacterium is attributed into 4 phylogenetic groups A, B1, B2 and D that differ from each other's in their virulence and disease caused by them. The current study examined the serotyping of *E. coli* strains isolated from milk and milk products with investigation of their phylogenetic grouping. Eight strains isolated from raw milk, Kariesh cheese, feta (white) cheese, and Ice cream (two strains from each product) were examined serologically by rapid diagnostic *E. coli* antisera sets and the result showed the presence of three serotypes O114:K90 (two strains), O128:K67 (three strains) and O55:K59 (three strains). Their phylogenetic grouping came out triplex PCR depend on the amplification of *chu*A, *yja*A, and the DNA fragment of *tsp*E4.C2.It showed that six strains belonged to the B2 group, and two strains belonged to D group based on the presence or absence of these genes.

1. INTRODUCTION

E. coli is a Gram-negative, facultatively anaerobic bacteria of family Enterobacteriaceae (Williams et al., 2010). *E. coli* strains are commensal germs that live in humans' and other mammals' intestines. Several groups of *E. coli* that distributed through contaminated milk and dairy products are pathogenic (Parseelan et al., 2018; Ombarak et al., 2019) which may occur through fecal contamination or unsanitary condition (Garbaj et al., 2016; Lara et al., 2016) so, it represents a sign for fecal contamination.

Based on the mechanism of illness, *E. coli* strains can be divided into six classes called pathotypes. *E. coli* strains that are enteropathogenic (EPEC), attaching and effacing, enterotoxigenic (ETEC), entero-invasive (EIEC), enterohemorrhagic(EHEC), and enteroaggregative (EAEC). Vero Toxin-Producing *E. coli*, Shiga-toxigenic *E. coli*, and Enterohaemorrhagic *E. coli* are names given to the *E. coli* strains that manufacture six toxins (Asmelash, 2015; Saba et al., 2015).

E. coli strains can be categorized into different phylogenies based on their genetic sub-culture and according to scientific studies, *E. coli* strains from different phylogroups exhibited various phenotypic and genotypic characters (Tenaillon et al., 2010). Numerous techniques are used to determine *E. coli* phylogroup, the Clermont triplex PCR phylogroup technique, a PCR-based test created by Clermont et al. (2000), is a quick method designed for categorizing *E. coli* strains into major phylogroups A, B1, B2, and D. This approach was applied to three *E. coli* genes: (i) the *chu*A gene, which is required for haem transport in *E. coli* O157:H7; (ii) the *tsp*E4.C2 DNA sequence, which is contained inside the gene encoding a putative lipase esterase; and (iii) *yja*A gene, which is a potential protein-producing gene (Doumith et al., 2012). Phylogenetic grouping of *E. coli* is vital not only for differentiation of *E. coli* strains, but also for determination the relationship between strains and illness caused by them (Halaji et al., 2022).

The following study aimed to group the strains isolated from milk and milk products serologically and phylogenetically.

2. MATERIAL AND METHODS

2.1. Method of isolation.

The bacteriological examination occurs by the traditional method according to APHA (2004). Then streak a loopful from the prepared samples into MacConkey's agar plates and incubate for 24 hours at 37ºC. Suspected lactose fermented colonies were picked up and streaked on the following media: Eosin methylene blue media (EMB); and TBX agar then incubated for another 24-48 hours at 37ºC, suspected colonies (colonies with metallic green sheen on EMB; and blue colonies on TBX agar) were picked up and confirmed as E. coli by using biochemical tests.

2.2. Bacterial strains

Eight *E. coli* strains isolated from milk and milk products.

2.2.1. Serological identification

Serotyping of *E. coli* isolates was achieved by using rapid diagnostic *E. coli* antisera sets (Anti-Coli, Sifin- Germany) obtained from Animal Health Research Institute, Dokki, Egypt, and used for lab diagnosis of pathogenic *E. coli* using

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the antisera reported in table (1) and by the technique of slide agglutination test previously described by Markey *et al.* (2013)

Table 1 Antisera used in serological identification of *E. coli.*

Polyvalent Sera	Contains antibodies against
Anti-coli I	O26:K60; O44:K74; O114:K90; O125:K70; O142:K86; O158:K-
Anti-coli II	O55:K59; O86:K61; O91:K-; O111:K58; O119: K69; O126:K71; O127:K63; O128:K67
Anti-coli III	O25: K11; O78:K80; O103:K-; O118:K-; O124:K72; O145:K-; O157:K-; O164:K-

2.2.2. Polymerase chain reaction

2.2.2.1. Extraction of DNA: occurs according to QIAamp DNA mini kit instructions.

2.2.2.2. Detection of examined genes by using PCR:

The PCR mixture was made up of 12.5 l Emerald Amp GT PCR master mix (Takara, Japan), 5.5 l PCR grade water, 1 l Forward primer (20 pmol), 1 l Reverse primer (20 pmol), and 5 l Template DNA till the total volume reached 25 l. Table (2) showed how the examined genes (*chu*A, *yja*A, and *tsp*E4C2) were amplified using specified primers. The reaction was then injected into a thermocycler using an applied bio system 2720.

Table 2 Oligonucleotide primers sequences Source: Metabion (Germany).

Gene	Sequence	Amplified product	Reference
chuA	GAC GAA CCA ACG GTC AGG AT TGC CGC CAG TAC CAA AGA CA	279 bp	Jeong et al., 2012
yjaA	TGA AGT GTC AGG AGA YGC TG	211 bp	
tspE4C2	ATG RAG AAT GCG TTC CTC AAC GAG TAA TGT CGG GGC ATT CA CGC GYC AAC AAA GTA TTR CG	152 bp	

After the cycling condition, the ladder was directly loaded into Agarose gel electrophoreses according to the instruction of Sambrook *et al*. (1989). The gel photographed using gel documentation system, data was analyzed using software (Automatic Image Capture Software, Protein Simple formerly Cell Biosciences, USA).

3. RESULTS

3.1. Serological identification

The serological identification of eight strains from the isolates (two strain from each product) typed them into three serotypes: O114:K90 (two strains), O128:K67 (three strains), and O55:K59 (three strains) (Table 3).

Table 3 Serodiagnosis of *E. coli* strains

	Samples	Serotyping	Strain grouping
1.	Raw milk	O114:K90	EIEC
2.	Raw milk	O128:K67	ETEC
3.	Kariesh cheese	O128:K67	ETEC
4.	Kariesh cheese	O55:K59	EPEC
5.	Feta cheese	O55:K59	EPEC
6.	Feta cheese	O128:K67	ETEC
7.	Ice cream	O55:K59	EPEC
8.	Ice cream	O114:K90	EIEC

3.2. Phylogenetic grouping of the isolated *E. coli* strains

The presence or absence of the three DNA fragments was used to perform phylogenetic grouping. (*chu*A, *yja*A, *tsp*E4.C2). The 8 *E. coli* isolates found were included in two phylogenetic groups: Group B2 (6/8) and Group D (2/8) (Table 4). PCR showed the presence of *chu*A gene (Fig. 1) and *tsp*E4c2gene (Fig. 2) in all eight strains. While *yja*A gene was only detected in six *E. coli* strains (Fig. 3).

Table 4 Phylogenetic analysis of the isolated strain

Sample	*chuA*	*yjaA*	*tspE4c2*	Phylogenetic group
1	+	-	+	D
2	+	+	+	B2
3	+	+	+	B2
4	+	+	+	B2
5	+	+	+	B2
6	+	+	+	B2
7	+	+	+	B2
8	+	-	+	D

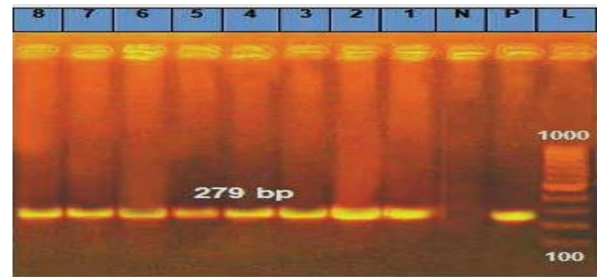

Fig. (1)Electrophoretic gel imaging of PCR for *E. colichu*A gene (279-bp).
Lane L: ladder (100-1000 bp), Lane P: control positive, Lane N: Control negative. Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute. Lanes from 1 to 8 are positive for the presence of the*chuA* gene.

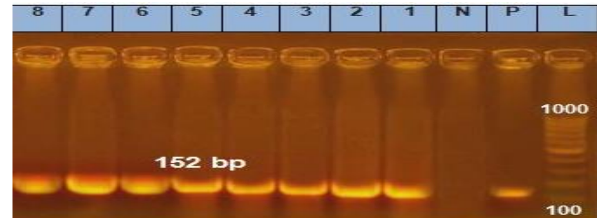

Fig. (2)Electrophoretic gel imaging of PCR for E. colitspE4c2gene (152-bp).
Lane L: ladder (100-1000 bp), Lane P: control positive, Lane N: Control negative. Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute. Lanes from 1 to 8 are positive for the presence of the tspE4c2 gene.

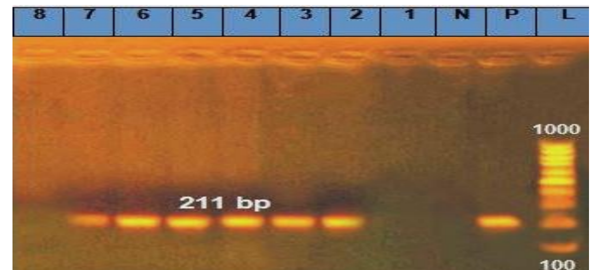

Fig. (3)Electrophoretic gel imaging of PCR for E. coliyjaA gene (211-bp).
Lane L: ladder (100-1000 bp), Lane P: control positive, Lane N: Control negative. Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute. Lanes from 2 to 7 are positive for the presence of theyjaAgene. While lanes 1 and 8 are negative for the presence of theyjaA gene.

4. DISCUSSION

Because *Escherichia coli* is generally found in the gastrointestinal tracts of animals, its isolation from milk and its products reflected evidence of either direct or indirect fecal contamination. It has public health implications since it causes several gastrointestinal illnesses, such as loose bowel syndrome in children and food poisoning. In addition to financial losses, milk and milk products contaminated with *E. coli* may proliferate easily on a number of substrates and deplete a variety of carbohydrates and organic components so render them unsellable during storage or even incapable for feeding in humans (Hassan *et al.,* 2021).

E. coli's outer membrane is composed of core oligosaccharides known as lipopolysaccharides (LPS), which comprise lipid A, and a unique polysaccharide known as O-antigen. Any flaw in O-antigens causes pronounced pathogenicity, indicating their relevance in host-pathogen interactions (Sarkar *et al.,* 2014). As a result of antigenic variety among the many O-antigens, they targeted as biomarkers for *E. coli* organization since 1940s (Markey *et al.,* 2013)

Results in Table (5) determined the serotyping of *E. coli* in examined strains that revealed the presence of O114:K90 and O128:K67 in raw milk samples, and this came nearly agreed with Abd El-Maabud (2014) who isolated *E. coli*O26

and O128 from raw milk; El nahas et al., (2015) isolated O114: H21, O111:H4, O26, and O127:H6 from raw milk samples and Ibrahim et al., (2019) illustrated presence of *E. coli*O26 : H11, O44 : H18, O55 : H7, O91 : H21, O111 : H2, O119 : H6, O121 : H7, O124, O127 : H6, O128 : H2, O146: H21, and O153 : H2 in raw milk and dairy products. SerotypesO128:K67 and O55:K59 were detected from samples of both Kariesh and white feta cheese, that came in harmony with Soukayana (2012) who illustrated *E. coli* serotypes O86, O55, O111; Also, El nahas et al., (2015) isolated *E. coli* serotypes O114:H21, O111:H4 O26, O127:H6 O119:H6 and O55 from Kariesh cheese; Ibrahim et al., (2019) and Hassan et al., (2021) who isolated *E. coli* types O18 (25%), O55: H7(21.8%), O114:H21(9.4%), O158(18.8%), O125:H21(9.4%) and O153:H45(15.6%) for Kareish cheese. While that isolated from Ice cream samples was found to beO55:K59 and O114:K90 *E. coli* strains that come nearly to the results recorded by Dalal (2012)who found O86, O55, O11 in ice cream samples and also Hassan et al., (2021).

E coli strains were allocated to one of the following phylogenetic groups: A, B1, B2, or D (Herzer et al., 1990). These phylogroups differ from each other in their genome size, with A and B1 strains having smaller genomes than B2 or D strains (Bergthorsson and Ochman, 1998). Strains from phylogroups B2 and D contained more virulency factors than strains from phylogroups A and B1 (Johnson et al., 2001). Groups B2 and D primarily contained aggressive strains that led to intestinal infections, whereas Groups A and B1 contained symbiotic and diarrhea-causing bacteria. (Clermont et al., 2000).

Results in this study revealed presence of six strains belonging to the B2 phylogroup and two strains belonging to the D phylogroup as recorded in Table (4). This contamination may come from poor hygienic measures or disseminated from the infected udder, which came in agreement with Dogan et al., (2006); Fernandes et al., (2011) and Suojala et al., (2011), who demonstrated that small percentage of strains isolated from mastitic milk belonged to groups B2 and D; Guerra et al., (2018) mentioned 10% of the isolates from mastitic milk was related to pathogenic phylogroups B2 (6%) and D (4%) ; Also, Jung et al., (2021) isolated the phylogroups B2 and D from bulk tank milk. While disagreed with Liu et al., (2014); Zhang et al., (2018) and Cruz-Soto et al., (2020) who mentioned *E. coli* was related to bovine mastitis mainly belonged to phylogenetic groups A and B1.This supports the theory that the topographical influenced on population structure of *E. coli* that isolated from different sources (Carlos et al., 2010).

5. CONCLUSIONS

Although *E. coli* was isolated from milk and milk products, different serotypes were detected and by examining their phylogenetic grouping found that they related to B2 and D groups. Therefore, strict hygiene guidelines should be maintained, and avoid buying from unknown sources.

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