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, Kanesh 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 O114K90 (two strains), O128K67 (three strains) and O55K59 (three strains). Their phylogenetic grouping came out triplet PCR depend on the amplification of chua, yjaA, and the DNA fragment of tspE4C2. 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.

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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, entero-toxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagie(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 chua gene, which is required for haem transport in *E. coli* O157:H7; (ii) the tspE4C2 DNA sequence, which is contained inside the gene encoding a putative lipase esterase; and (iii) yjaA gene, which is a potential protein-producing gene (Douflhet 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
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.

<table>
<thead>
<tr>
<th>Polyvalent Sera</th>
<th>Contains antibodies against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-chuA I</td>
<td>O114:K90; O144:K47; O114:K90; O125:K70; O125:K90; O55:K5; O55:K70; O68:K1; O111:K90; O119:K60; O128:K27; O127:K35; O128:K67</td>
</tr>
<tr>
<td>Anti-chuA II</td>
<td>O25:K1; O76:K60; O105:K1; O118:K70; O124:K72; O145:K5; O157:K1; O164:K6</td>
</tr>
<tr>
<td>Anti-chuA III</td>
<td>O55:K59; O86:K11; O91:K61; O91:K80; O103:K80; O124:K72; O108:K75; O128:K67</td>
</tr>
</tbody>
</table>

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 (chuA, yjaA, and tspE4C2) 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).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>chuA</td>
<td>GAC GAA CCA ACC AGT AGG AT</td>
<td>279 bp</td>
<td>Jeong et al. 2012</td>
</tr>
<tr>
<td>yjaA</td>
<td>TGG CCG CAG TAC CAA AGA CA</td>
<td>211 bp</td>
<td></td>
</tr>
<tr>
<td>tspE4C2</td>
<td>ATG RAG AAT GCG TTC CTC AAC</td>
<td>152 bp</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Serotyping</th>
<th>Strain grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>O114:K90</td>
<td>EIEC</td>
</tr>
<tr>
<td>Raw milk</td>
<td>O128:K67</td>
<td>ETEC</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>O128:K67</td>
<td>ETEC</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>O55:K59</td>
<td>EPEC</td>
</tr>
<tr>
<td>Feta cheese</td>
<td>O55:K59</td>
<td>EPEC</td>
</tr>
<tr>
<td>Feta cheese</td>
<td>O128:K67</td>
<td>ETEC</td>
</tr>
<tr>
<td>Ice cream</td>
<td>O55:K59</td>
<td>EPEC</td>
</tr>
<tr>
<td>Ice cream</td>
<td>O114:K90</td>
<td>EIEC</td>
</tr>
</tbody>
</table>

3.2. Phylogenetic grouping of the isolated E. coli strains

The presence or absence of the three DNA fragments was used to phylogenetically grouping. (chuA, yjaA, tspE4C2). 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 chuA gene (Fig. 1) and tspE4C2 gene (Fig. 2) in all eight strains. While yjaA gene was only detected in six E. coli strains (Fig. 3).

Table 4 Phylogenetic analysis of the isolated strain

<table>
<thead>
<tr>
<th>Sample</th>
<th>chuA</th>
<th>yjaA</th>
<th>tspE4C2</th>
<th>Phylogenetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B2</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>D</td>
</tr>
</tbody>
</table>

Fig. (1) Electrophoretic gel imaging of PCR for E. coli chuA gene (279 bp).
Fig. (2) Electrophoretic gel imaging of PCR for E. coli yjaA gene (152 bp).
Fig. (3) Electrophoretic gel imaging of PCR for E. coli tspE4C2 gene (211 bp).

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. coliO26

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 (Berghorson and Ochman, 1998). Strains from phylogroups B2 and D contained more virulence 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.

6. REFERENCES