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## Original Article

# Phylogenetic evaluation of *Escherichia coli* isolated from cases of bacillus diarrhea

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**Summary**

*Escherichia coli (E. coli)* is the normal flora of the gastrointestinal tract of humans and animals, although most of the strains are known not to be pathogenic. Pathogenic strains of *E. coli* can cause a wide variety of diseases, including urinary tract infection, intestinal and extra-intestinal diseases, as well as problems in the respiratory system. In fact, 80-90 % of urinary tract infections are attributed to *E. coli* bacteria along with different phylogenetic groups of these bacteria. The aim of this study was to determine phylogenetic groups of *E. coli* isolates from fecal samples of calves affected with Bacillus in the Moghan region, northwest of Iran. Samples were taken from 60 calves (1 to 30 days old) with common basil diarrhea in a dairy farm located in the Moghan region in the northwest of Iran in 2017. Samples were cultured in *E. coli* culture media. Among isolated bacteria, 50 samples (83.33%) were positive for *E. coli* bacteria. Then the samples were coded and prepared for PCR. The phylogenetic background of the isolates was determined according to the presence of the *chuA*, *yjaA*, and *TspE4.C2* markers in *E. coli* bacteria. The results showed that among 50 isolates, 31 were B2 group (62%), 8 were D group (16%), 3 were B1 group (6%), 1 was A group (2%), and remaining cases were 7 (14%). Obtained results clearly demonstrated that the most frequent phylogenetic group of *E. coli* was B2, whereas group A was the least one in the Moghan region.
***Keywords*: *E. coli*,** Phylogenetic, Bacil diarrhea, Calf, The Moghan

## Introduction

*Escherichia coli (E. coli)* is the normal flora of the gastrointestinal tract in most animals and humans. Pathogenic strains of *E. coli* can induce a wide variety of intestinal and extra-intestinal diseases including, sepsis, neonatal meningitis, gastroenteritis, and several infections, such as urinary tract infections (Khan et al., 2017; Morcatti Coura et al., 2015). Urinary tract infection is one of the most frequent bacterial complications, and the respiratory system is the second common reported infection. Several bacteria can cause infection in the urinary system in which *E. coli* is the most common one. In fact, *E. coli* is still the predominant microorganism in the urinary tract all over the world, which causes 80-90% of urinary tract infections (Lee et al., 2016). In vitro genome

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examination of *E. coli* strains in different phylogenetic groups revealed that specific genes or fragments of bacterial DNA could be used as specific markers for the phylogenetic classification of the *E. coli* strain (Coura et al., 2017). Three suggested specific markers consist of *chuA*, a necessary gene for the transference of enterohemorrhagic O157: H7; *yjaA*, detected in the genome of *E. coli* K-12 with anonymous function and *TSPE4.C2* an unknown DNA segment of the *E. coli* genome (Gordon et al., 2008). Based on a phylogenetic study, different strains of *E. coli* have been identified, having different phylo-groups (Coura et al., 2017). Until now, eight phylogenetic groups (A, B1 ,B2 , C, D, E, F, I ) have been recognized, and the most important phylogenetic groups are A, B1, B2, and D (Coura et al., 2017). Groups A and B1 are known as the sisters, while the B2 group is recognized as a common ancestor of mentioned groups which differs in many features such as living conditions (Alonso et al., 2016), using different carbon sources, resistance to antibiotics, growth rate and pathogenicity (Guardabassi et al., 2004). It has been reported that the most adaptable strain of *E. coli* in the environment belongs to the B1 group (Walk et al., 2007; Alonso et al., 2016). Some evidence of variable genome size are existed in *E. coli* strains in which groups A and B1 have smaller genomes than B2 and D. It is noteworthy to mention that the extraintestinal pathogenic *E. coli* (ExPEC) bacteria often belong to B2 and D groups, while commensal strains often belong to A and B1 groups and intestinal pathogenic strains often belong to A, B1 and D groups (Alonso et al., 2016). For the first time, Clermont (2000) utilized the PCR method to evaluate 230 strains of *E. coli*, consisting of *chuA* and *yjaA* genes and *TSPE4.C2* fragments, based on previously described methods (Ribotyping and MLEE) (Clermont et al., 2000). Since other techniques need more complex and time-consuming procedures requiring a set of model strains, PCR is used as a simple and fast method with higher sensitivity and specificity (Gordon et al., 2008). Bacteria show different features based on host niche, pathogenicity, antibiotic resistance, and, more importantly, specific virulence factors (Shaheen et al., 2013) hence, there is a need to identify *E. coli* sub-groups for developing a potential treatment for related diseases. Thus, we aimed to discover the main source of environmental pollution, such as water resources (Rzewuska et al., 2015), in order to treat *E. coli* infections.

## Materials and methods

### *Isolation and identification of E. coli*

Fifty rectum (anus) samples were obtained from 60 calves with colibacillosis diarrhea in Moghan dairy farm located in the Moghan Agro-industry Complex region in 2017. After sampling and primary cultures in MacConkey agar (Merck, Germany), we used TSA (Merck, Germany) and EMB agar media (Merck, Germany), and IMViC (Merck, Germany) tests for final isolation and identification of *E. coli* samples. After that, the isolates were coded and kept in nutrient broth with 15% glycerol at –20 °C for subsequent experiments.

### *DNA extraction*

The obtained colonies from each sample were dissolved in the TAE (Tris, acetate, and EDTA) buffer (Arvin Shimi, Iran). In brief, tubes were placed in a boiling water bath for 25 minutes by shaking every 5 minutes till well dissolved and centrifuged for 12 minutes at 10,000 rpm. The supernatant containing bacterial DNA was collected and kept in the refrigerator for 10-15 minutes. The concentration and purity of extracted bacterial DNA were measured using a Nano-drop spectrophotometer (Thermo Scientific, USA). The DNA samples were kept in a freezer until being used (Coura et al., 2017; Sobieszczańska, 2008).

### *Polymerase chain reaction (PCR)*

The Multiplex PCR method was applied to verify and classify the phylogenic strains of *E. coli*. The PCR reaction mixture contained 12.5 µl Master mix (containing TAE buffer 1 M, Taq DNA polymerase, Mgcl2, and DNTP), as well as 0.5 µl of each primer (*ChuA*, *yjaA* genes and *TSPE4.C2*

fragment), 2 µl of concentration adjusted DNA template, and finally adding water to a final volume 25 µl. (Table 1). The amplification took place as follows: Initial denaturation step 5 minutes at 95 ◦c which followed by 40 cycles of denaturation at 94 ° C for 30 seconds, annealing step 57 ° C for 30 seconds, extension 72 ° C for 30 seconds, and final extension at 72 ° C for 5 minutes (Coura et al., 2017; Sobieszczańska, 2008). Primer pairs were designed using oligo 7 Primer Software to obtain the amplicons ranging from 281, 216, and 152bp, as depicted in Table 1.

### *DNA Electrophoresis with agarose gel*

The PCR products were analyzed on gel electrophoresis (1%) in order to identify *chuA* and *yjaA* genes and the *TSPE4.C2* fragment. Sample without DNA served used as a negative control (Laboratory nonpathogenic E. coli strain MG1655). After electrophoresis, the PCR products visualized by UV transilluminator (BioDocAnalyze; Biometra) based on presence or absence of genes and DNA fragment as: B2 group (*yjaA* + '*chuA*), B1 group (*TspE4.C2* + '*chuA*_'), D group (*TspE4.C2* + '*chuA* +) and group A (TspE4.C2-' *chuA*-) (Table 2) (Coura et al., 2017; Sobieszczańska, 2008) .

## Results

A total of 50 *E. coli* strains from feces of calves with colibacillosis diarrhea were allocated into three phylogenetic groups (i.e., A, B2, and D) and six subgroups (i.e., A0, A1, B22, B23, D1, and D2). According to multiplex PCR-based phylotyping obtained results, group B2 was the majority of the isolates (n=31, 62%), D (8 isolates, 16%), B1 (3 isolates, 6%), A (1 isolate, 2%), and unknown group (7 isolates, 14%) (Table 3 & Fig. 1). According to the results, phylogenetic group B2 showed a higher frequency of *E. coli* genetic markers (Table 4).

**Table 1.** The primer sets, sequences, and product length.

| Primer | Primer Sequence (5'-3') | Product Length (bp) |
|---|---|---|
| ***chuA* (F)** | ATGATCATCGCGGCGTGCTG | |
| ***chuA* (R)** | AAACGCGCTCGCGCCTAAT | **281** |
| ***yjaA* (F)** | TGTTCGCGATCCTTGAAAGCAAACGT | |
| ***yjaA* (R)** | ACCTGTGCAAACCGCCTCA | **216** |
| ***TSPE.C2* (F)** | GCGGGTGAGACAGAAACGCG | |
| ***TSPE.C2* (R)** | TTGTCGTGAGTTGCGAACCCG | **152** |

**Table 2**. Identification and phylogenetic analysis of *E. coli* strains using PCR technique.

| Phylogenetic marker / Group | A | B1 | D | D | B2 | B2 |
|---|---|---|---|---|---|---|
| ***chuA*** | - | - | + | + | + | + |
| ***yjaA*** | + | - | - | - | + | + |
| ***TSPE4.C2*** | - | + | + | + | - | + |

**Table 3.** Distribution of the *E. coli* phylogenetic subgroups

| Phylogenetic subgroup | Value | Percentage |
|---|---|---|
| **A** | 1 | 2 |
| **B1** | 3 | 6 |
| **B2** | 31 | 62 |
| **D** | 8 | 16 |
| **Other** | 7 | 14 |
| **Total** | 50 | 100 |

**Table 4.** Distribution of *E. coli* genetic markers

| | A | B1 | B2 | D | Other |
|---|---|---|---|---|---|
| **CHU** | 0 | 0 | 31 | 8 | 0 |
| **TSPE4** | 0 | 3 | 28 | 8 | 7 |
| **YJA** | 1 | 0 | 31 | 0 | 7 |

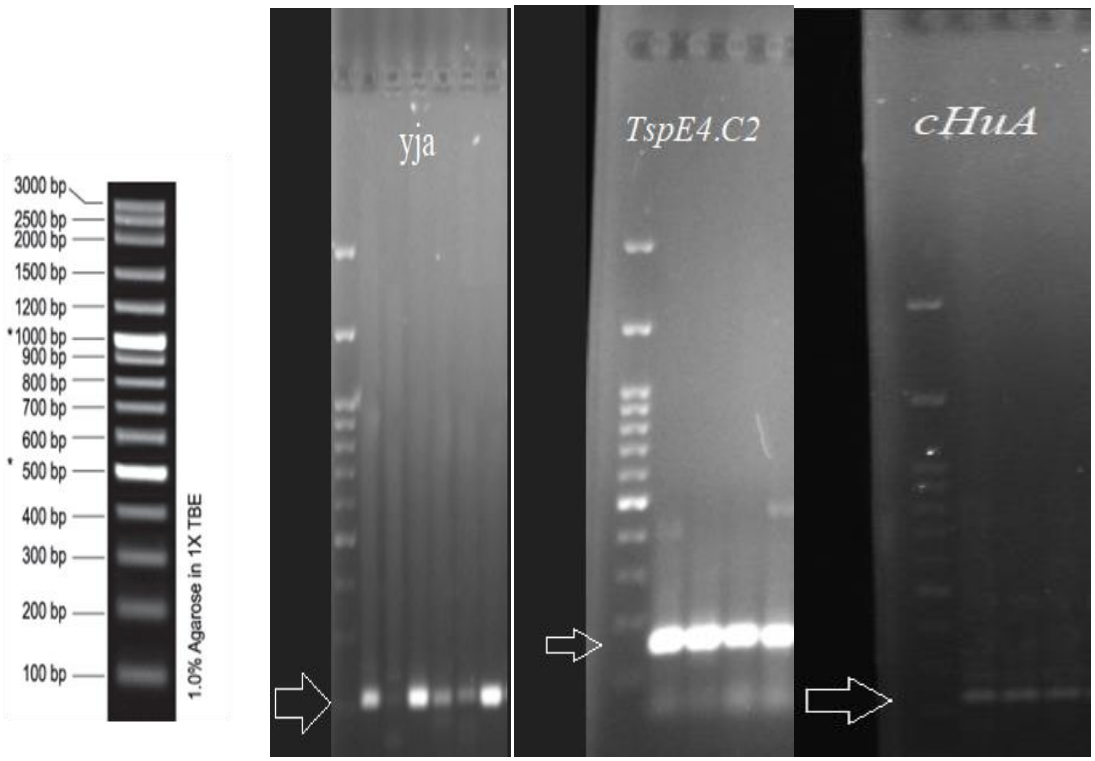


**Fig. 1.** Agarose gel electrophoresis for the analysis of phylogenetic groups. Amplicon size are 281bp (*chuA*), 216 bp (*yjaA*), and 152bp (*TSPE.C2*).

## Discussion

Cattle, poultry, and other food-producing animals are an important host for *E. coli* (Morcatti Coura et al., 2015). Certain *E. coli* strains have been associated with neonatal diarrhoea in ruminants which causes considerable economic losses in the dairy industry all around the world (Shahrani et al., 2014). To date, there have been very few published studies on phylogenetic grouping of E. coli in Iran (Ghanbarpour and Oswald, 2010). In this line, several evidence have shown that *E. coli* can be the

cause of a variety of diseases, including intestinal and extra-intestinal problems (Nakhaee et al., 2015). The most common classification for ExPEC strains are groups B2 and D. The commensal strains belong to A and B1 as well as the other six subgroups of A0, A1, B22, B23, D1, and D2. Obtained results are in accord with clinical findings, and also, it has been demonstrated a strong relationship between phylogeny and virulence factors of bacteria (Derakhshandeh et al., 2014; Escobar-Páramo et al., 2004; Khan et al., 2017). Based on obtained results from the current study, two phylogenic groups B2 and D showed the predominant population of isolated samples (62 and 16 percent, respectively). Since these groups belong to pathogenic bacteria, and with regard to samples collected from calves with diarrhea, therefore this result is the complete analogy with previously published results (Barzan et al., 2017; Derakhshandeh et al., 2014; Maciel et al., 2019). In addition, it has been reported that in healthy food-producing animals, such as cattle, the most common group is B1 while, A group is frequent in pigs and chickens (Escobar-Páramo et al., 2004; Lee et al., 2016). These findings are consistent with our results. Indeed, the B2 group is rarely found in healthy cattle, chickens, and pigs (Lee et al., 2016). Host habitat, diet, gut morphology, body mass and climate are the important key factors in the distribution of the *E. coli* groups among all mammalian hosts (Gordon & Cowling, 2003). Moreover, it has been found that some *E. coli* strains can be adapted to the gut niche (Jubelin et al., 2018). Carlos et al. (2010) illustrated that some animals such as cows, goats, and sheep were rarely express the *chuA* and *yjaA* genes, whereas these are common genes in humans, chickens, and pig (Lee et al., 2016). In another similar study, Sobieszczaeska (2008) revealed that 95.5% of enter aggregative *E. coli* strains carry the *chuA* gene (Sobieszczańska, 2008). Overall, based on the presence of the *chuA*, *yjaA*, and *TspE4.C2* markers, we found that phylogenetic group B2 is more frequent among all isolated samples.

## Conclusion

Through the current research, we can conclude that among isolated samples, the most frequent phylogenetic group was B2, and the least one was group A in the Moghan region.

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**Conflict of Interest Statement**

The authors declared there is no conflict of interest.

**Ethical approval**

Not applicable

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