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Short Communication

Phylogenic typing of *Escherichia coli* isolated from broilers with collibacillosis in Tabriz, North West of Iran

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ABSTRACT

In this study, to know about the phylogeny of *Escherichia coli* isolated from broilers with collibacillosis in Tabriz, 70 *E. coli* isolates recovered from broilers with collibacillosis were characterized for phylogenetic group (A, B1, B2, D) by multiplex PCR. Of the all 70 samples, 35 (50%) isolates were classified as type A, 32 (45%) as type D, 2 (2.8%) as type B1 and 1 (2.8%) as type B2. This study demonstrates the high prevalence of *E. coli* types A and D in infected broilers. This shows that the collibacilosis-causing *E. coli* bacteria are typical commensals, type A alongside pathogenic type, D in Iran. It is possible that this type of *E. coli* could acquire virulence genes from pathogenic types. Of course, such a claim needs further study.

Keywords: Escherichia coli, phylogeny, collibacillosis, Tabriz, broiler

INTRODUCTION

Escherichia coli strains cause a number of diseases in domestic poultry. Among these diseases is a severe systemic form termed colisepticaemia, which is characterised by the presence of E. coli in the blood, and colonisation of organs including the heart, liver and spleen (Barnes *et al* 2003). Avian pathogenic *Escherichia coli* (APEC) are the leading pathogenic agents in the infections of extra intestinal tissues in chicken (Gross 1991). The initial infection primarily occurs in the respiratory tract and ultimately results in diverse diseases (Dho & Lafont 1999). The condition is commonly characterized by air sacculitis and may involve septicemia with pericarditis, perihepatitis, and salpingitis (Gross 1994).

Early Multi-Locus Enzyme Electrophoresis (MLEE) studies demonstrated the existence of subspecies in *E. coli*. More extensive MLEE analysis, together with other techniques of DNA analysis, confirmed the existence of subspecies. Traditionally, four recognized subspecies or phylo-groups of *E. coli* have been designated A, B1, B2, and D. Clermont et al. (2000) described a multiplex PCR protocol that determines the

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presence/absence of two genes (chuA and yjaA) and an anonymous DNA fragment (TSPE4.C2). The presence or absence of these three PCR products is used in the manner of a dichotomous key to assign an unknown isolate to one of the four main phylo-groups of *E. coli* (Clermont *et al* 2000). The mechanisms of pathogenesis in the avian pathogenic *E. coli* (APEC) have not been thoroughly understood, however, the correlation of pathogenicity with virulence factors has been recently extensively explored (Chouikha *et al* 2008, Dziva & Stevens 2008). The aim of this study was to investigate the phylogenetic groups of *E. coli* isolated from broilers with collibacillosis in Tabriz, North West of Iran.

MATERIALS AND METHODS

Bacterial isolates. Seventy *E. coli* isolates were recovered from broilers with collibacillosis in Tabriz, North West of Iran from different farms and were confirmed according to the biochemical standards described previously (Quinn *et al* 1994).

DNA extraction. A colony from BHI (Brain heart infusion) agar was transferred to a clean microtube and 500ml lysis buffer (pH 8, containing 5 mol NaCl, 100 mmol Tris-base, 20 mmol EDTA-Na2 and CTAB 20%) was added. The samples were then incubated at 60-65 °C for 10 min. and centrifuged at 12,000 x g for 10 min. The pellet was resuspended in chloroform-isoamyl alcohol (24:1), and centrifuged for one min. 12,000 x g. Then, the pellet was resuspended in cold isopropanol and transfer to refrigerator for 30 min. Next, 70% ethanol was added to supernatant and centrifuged for one min. 12,000 x g. Finally, 50µl TE-buffer was add to the pellet and stored as DNA template.

Multiplex PCR for Phylogenic typing of E. Coli. The phylogenic type was determined by multiplex PCR (Clermont *et al* 2000). Each 25 µl reaction contained 0.2 µl DNA template, 1 U Taq DNA polymerase, 3.2 mM from primer (Table 1), 200 µM of each dNTP, 4 µl of 1 x PCR buffer, and 2 mM $MgCl_2$. The PCR reactions were intiated by an initial denaturation at 94 °C for 5 min followed by 35 cycles, each cycle consisting of DNA denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec. The cycles were followed by a final extension at 72 °C for 7 min. Amplified PCR products were separated by 1% agarose electrophoresis and finally stained with ethidium bromide and photographed.

Table 1. Specific primer for determinate phylogenic groups using multiplex PCR

Gene	Name	Sequence (5'-3')	Size band
chuA	ChuA.1	GACGAACCAACGGTCAGGAT	279
	ChuA.2	TGCCGCCAGTACCAAAGACA	bp
yjaA	YjaA.1	TGAAGTGTCAGGAGACGCTG	211
	YjaA.2	ATGGAGAATGCGTTCCTCAAC	bp
TSPE4.C2	TspE4C2.1	GAGTAATGTCGGGGCATTCA	152
	TspE4C2.2	CGCGCCAACAAAGTATTACG	bp

RESULTS AND DISCUSSION

Phylogenic typing of *E. coli* was conducted based on amplification of 3 different phylogenic groups using multiplex PCR in the presence of each specific primer. The phylogenic group of each strain was determined as follows; Chu A (+), Yja A (+), TSPE4.C4 (+/-) was assigned to group B2; Chu A (+), Yja (-), TSPE4.C4 (+/-) was assigned to group D; Chu A (-), Yja A (+/-), TSPE4.C2 (+) was assigned to group B1; and chu A (-), Yja A (+/-), TSPE4. C2 (-) was assigned to group A (Figure 1 and 2). Of the all 70 samples, 35 (50%) isolates were classified as type A, 32 (45%) as type D, 2 (2.8%) as type B1 and 1 (1.4%) as type B2 (Table 2).

Table 2. Results of phylogenic typing of isolates of *Escherichia coli*

Phylogenic type	No. of type	Percentage
A	35	50
B1	2	2.8
B2	1	1.4
D	32	45
Total	70	100

This is the first report describing phylogenic typing of *E. coli* isolated from broilers with collibacillosis in Northwestern Iran. The result of this study and other similar reports conducted in different regions of Iran, revealed that types A and D are the common

phylogenic types isolated from broilers with collibacillosis in this country (Ghanbarpour *et al* 2011). Phylogenetic grouping of *E. coli* has been used in epidemiological virulence studies (Picard *et al* 1999, Russo & Johnson 2000). Phylogenic type A consists mainly of strains that are commensally orientated and not essentially pathogenic for a healthy host (Clermont *et al* 2000).

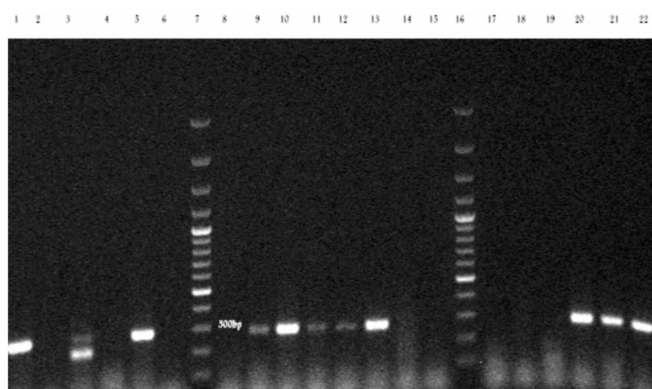

Figure 1. Phylogenic typing by PCR of the isolates. Lane 7 and 16: 100 bp DNA marker, Lane 1, 5, 9 to 13 and 20 to 22: type D. Lane 2, 4,6,8,14,15 and 17 to 19: type A, Lanes 3: type B2.

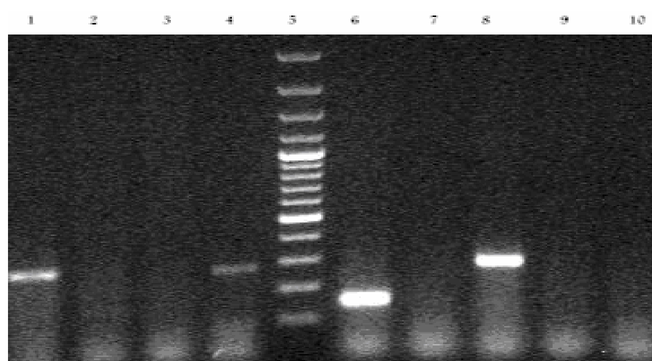

Figure 2. Phylogenic typing by PCR of the isolates. Lane 5: 100 bp DNA marker, Lane 1, 4, 8: type D. Lane 2, 3, 7, 9 and 10: type A, Lanes 6: type B2.

Our results show higher prevalence of this genogroup in infected broilers. This demonstrates that the *E .coli* bacteria causing collibacilosis are typical commensals in Iran. It may be because this type acquires virulence genes from pathogenic types. Phylogenetic analyses have shown potentially pathogenic strains belong mainly to B2 and D groups. A diverse range of bacterial virulence factors have been postulated in promoting avian disease, including adhesins, iron acquisition systems, hemolysins, antibactericidal factors and toxins (Dias da Siveira *et al* 2002, Ewers *et al* 2003, Parreira & Gyles 2003). Intestinal or extraintestinal *E. coli* infections are associated with strains possessing numerous virulence factors located on plasmids, bacteriophages, or the bacterial chromosome (Mu uhldorfer & Hacker 1994). Several studies have demonstrated that pathogenic *E. coli* strains may be derived from commensal strains through acquisition of chromosomal or extra-chromosomal virulence operons (Finlay & Falkow 1997, Ochman *et al* 2000). Some other mechanisms such as 'black hole' genomic deletions or random functional point mutations (Sokurenko *et al* 1998 Maurelli *et al* 1998) may also be strategies by which a commensal strain may become virulent. This statement needs more investigations on environmental and host conditions including dosage of organism, the status of immune systems associated with bacterial virulence factors (Barnes *et al* 2003). Further studies of virulence factors among these regional isolates may be needed to elucidate the virulence profile of avian *E. coli* and to establish exactly the mechanism of transmission between pathogenic or nonpathogenic strains in this region.

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