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## Differentiation of avian *Escherichia coli* isolates through RAPD PCR

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#### Abstract

This study involved thirty *E. coli* strains isolated from poultry birds from different poultry houses. Blood agar was used for inoculation at first followed by EMB and MacConkey agar for suspected samples only. 30 out of 100 samples where bacterial growth was observed were pinned down as *E. coli*. For the detection of genetic difference between *E. coli* strains, these strains were typed by random amplification of polymorphic DNA (RAPD). It was followed by amplification of DNA extracted from 30 strains of *E. coli* by RAPD primer (COL 1) which yielded eight different band profiles. In our study we found great genetic heterogeneity among different *E. coli* strains. The RAPD analysis, due to its simplicity and rapidity appears to be a highly valuable tool for studying *E. coli* molecular epidemiology.

**Keywords:** *Escherichia coli*, RAPD, poultry

#### Introduction

Infections with *Escherichia coli* are a major worry for the poultry industry<sup>[1]</sup>. It is commonly found in the digestive tracts of animals and birds on a regular basis, however not all *E. coli* isolates can result in pathological illness<sup>[2]</sup>. Many disease conditions like enteritis, pericarditis, perihepatitis, synovitis etc. are primarily caused by APEC strains of *E. coli* strains<sup>[5]</sup>. The bacteriological and serological techniques used for microbe typing is frequently restricted to a small number of specialized reference labs. However, due to sensitivity issues, these conventions seldom distinguish bacterial isolates properly<sup>[3]</sup>. Using techniques like multi-locus enzyme electrophoresis (MLEE)<sup>[14]</sup> pulsed-field gel electrophoresis (PFGE)<sup>[15]</sup>, random amplification of polymorphic DNA (RAPD)<sup>[16]</sup>, bacterial restriction endonuclease digest analysis (BRENDA)<sup>[10]</sup>, polymerase chain reaction (PCR)<sup>[17]</sup> that discover variations in the genetic make-up of a microbial community, *E. coli* isolates can be separated into genetically diverse isolates<sup>[4]</sup>.

The majority of APEC strains are indistinguishable from the typical commensals that live in birds' gastrointestinal tracts. Currently, the phenotypic distinction between pathogenic strains of *E. coli* is used in diagnosis of typical infection and nonpathogenic flora<sup>[6, 7]</sup>. The use of phenotypic differentiation techniques is not common in many clinical laboratories since they can be time-consuming and difficult. Numerous genotypic techniques have been demonstrated to be beneficial for species identification, epidemiological typing, and figuring out how closely related pathogenic and nonpathogenic bacteria are genetically<sup>[18, 19]</sup>.

The genetic makeup of *E. coli* strains can help identify those that are particularly harmful to a given host and provide information for epidemiological research on the places where infections occur and how diseases spread<sup>[10]</sup>. One innovative technique, RAPD analysis on arbitrarily primed PCR (APPCR) typing, is used in numerous studies using a molecular approach<sup>[8, 9, 13]</sup>. It is quick and reliable PCR-based genetic typing technique. The current study aimed to detect the genetic dissimilarities in *E. coli* isolates from local colibacillosis suspected birds.

#### Materials and Methods

A total of 100 visceral organ (liver heart, lungs) samples, were collected from different broiler farms and were stored at 4 °C immediately. It was followed by inoculation of samples on sheep blood agar and MacConkey agar.

The IMViC biochemical tests was used for identification and Brain Heart infusion broth with 25% glycerol was used for storage of *E. coli* at -70 °C. A random COL-1 primer or primer 4 (3'-AAGAGC CCG T-5') was used at a concentration of 1µM.

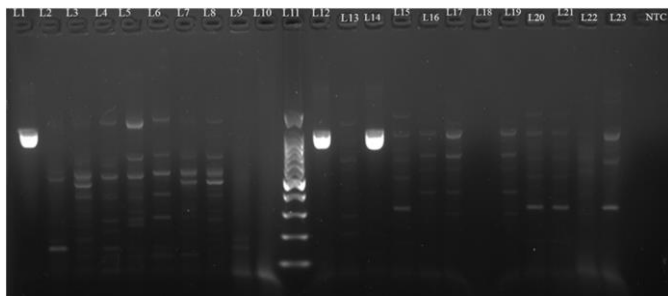
300µl distilled water was taken in an eppendorf tube followed by addition of selected *E. coli* colonies. The tube was mixed properly by vortexing followed by incubation at 98 °C for 10 min in boiling water bath and snaps chilled in ice for 4hr or overnight, then the boiled suspension centrifuged 10000 rpm for 10mins and supernatant collected in a separate eppendroff tube and stored at -20°C for further use.

For RAPD analysis, a total volume of 15µl reaction mixture was prepared with 2.5µl template DNA, 2x PCR Master mix (Takara) of 7.5µl, 1µl of RAPD primer, 4µl NFW. The samples were amplified through PCR conditions as 95 °C for 15mins, 35 cycles of 94 °C for 1min, 38 °C for 1min and 72 °C for 2 min, with a final 10 min elongation step at 72 °C.

The red safe (Intron, Korea) staining was used for visualization of RAPD products after 1.5% agarose gel electrophoresis.

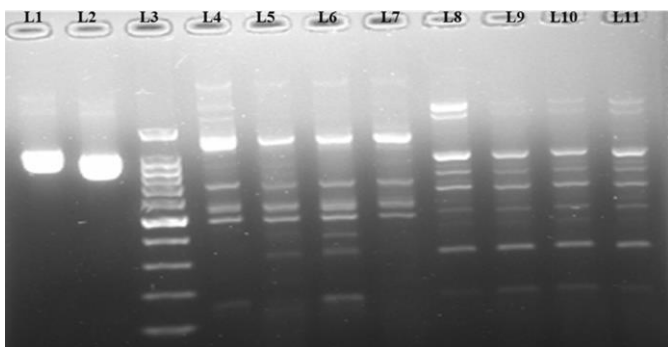
## Results and Discussion

A total of 30 (30%) *E. coli* isolated from 100 samples. The electrophoretic profiles produced by RAPD analysis using the COL-1 primer on *E. coli* isolates from chickens revealed eight different band profiles (Fig. 1 &2).



L1 to L10 and L12 to L23 are *E. coli* isolates  
L11: 100bp DNA Ladder

**Fig 1:** RAPD PCR for Avian *E. coli*



L3: 100bp Ladder  
L1 to L11 are *E. coli* isolates

**Fig 2:** RAPD PCR for Avian *E. coli*

Colibacillosis is an acute, mostly systemic disease brought on by infections with avian pathogenic *Escherichia coli* [12, 13]. It has a considerable negative economic impact on the global chicken industry. A complex condition known as avian colibacillosis causes several organ abnormalities, with airsacculitis, perihepatitis, and peritonitis being the most common. Avian pathogenic *E. coli* infections are influenced

by various factors like environmental factors, early viral infectionsetc [16].

The main aim of this study was to use RAPD to look into the genetic variations among avian *E. coli* isolates. Thirty *E. coli* strains obtained from chickens were heterogeneous which yielded 8 profiles that RAPD recognised. The method was able to classify different strains of *E. coli* successfully.

Maurer and co-workers (1998) [12] had observed sixteen different RAPD types in *E. coli* isolates where the random amplification of polymorphic DNA recognized eight different profiles. Similarly, Chansiripornchai *et al.* (2001) [3] found fifty random amplification of polymorphic DNA types where they had used two different primers in *E. coli* strains. Furthermore, it is observed that the random primer number 4 gave highest discriminatory power [3, 10]. The use of different and more than one random amplification of polymorphic DNA primers may improve differentiation power of RAPD process [3, 11]. Similarly, Cave *et al.* (1994) [2] found twenty-eight different patterns among 60 *E. coli* strains.

The study found out that avian *E. coli* aregenetically very heterogenic which showed similarity with works of Chansiripornchai *et al.* (2001) [3], and Maurer *et al.* (1998) [12]. There are chances that more than one *E. coli* type can be found from the same bird which has been reported in various studies [1, 11, 19]. Maurer *et al.* (1998) [12], also found certain *E. coli* RAPD types throughout the year. Furthermore, due to short lifespan of broilers, the birds may not have enough time to build immunity to the *E. coli* genetic type, which may result in encountering different *E. coli* kinds.

## Conclusion

The findings of this study showed that there is significant genetic variation among chicken *E. coli*isolates. Moreover, RAPD analysis has been suggested as an alternate method to identify *E. coli* isolates from avian sources. The complete genome is the goal for genotyping, which has demonstrated that the RAPD assay has good discrimination ability. To demonstrate the validity of this inference, more genotyping research using strains originating from other origins is required.

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