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Random Amplified Polymorphic DNA (RAPD) Analysis of *Escherichia Coli* Isolated From Chickens*

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In this study, 48 *E.coli* strains isolated from colibacillosis suspicious hens at different poultry houses were examined. These samples were inoculated on to blood agar and then suspicious colonies were transferred on to EMB and MacConcey agar. Bacterial growth was observed in 110 samples, 48 (13,6%) of which were identified as *Escherichia coli (E.coli)*. *E.coli* strains were typed by randomly amplified polymorphic DNA (RAPD) to detect the genetic differences between at *E.coli* strains in this region. Extracted DNA samples from 48 strains of *E.coli* were amplified by RAPD primer (COL 1). Nine different band profiles were obtained. These results indicated that there were great genetic heterogeneity between *E.coli* strains in this region. RAPD analysis had the highest discriminatory capacity for typing *E.coli* isolates. Because of its simplicity and rapidity, RAPD analysis appears to be a highly valuable tool for studying *E.coli* molecular epidemiology.

Keywords: *E.coli, RAPD, chicken, PCR.*

Tavuklardan İzole Edilen *Escherichia Coli* Suşlarının Random Amplified Polymorphic DNA (RAPD) Analizi İle Tiplendirilmesi

Bu çalışmada, colibacillosis şüpheli farklı kanatlı işletmelerinden 48 *E.coli* suşu izole ve identifiye edildi. Suşların kanlı agara ekimleri yapıldıktan sonra, EMB ve MacConkey agara ekimleri yapıldı.110 örneğin bakteriyel kültürü sonucunda 48 (%13,6)'i *E.coli* olarak identifiye edildi. Bu bölgedeki *E.coli* soyları arasındaki genetik farklılıkları tespit etmek için RAPD metodu uygulandı. 48 *E.coli* izolatından ekstrakte edilen DNA'lar RAPD primer (COL 1) ile çoğaltıldı. RAPD analizinden dokuz farklı profil elde edildi. Bu sonuçlar bu bölgedeki *E.coli* soyları arasında büyük bir genetik farklılık olduğunu gösterdi. RAPD metodunun *E.coli* soylarını tiplendirmede yüksek oranda ayırıcı olduğu görüldü. Sonuç olarak bu çalışma; RAPD metodunun hem kolay hem de daha hızlı olmasından ötürü, *E.coli*'nin moleküler epidemiyolojisinin çalışılmasında, yüksek oranda etkili bir metod olduğunu gösterdi.

Anahtar kelimeler: *E.coli, RAPD, tavuk, PZR. .*

Introduction

Esherichia coli infections are a significant concern to the poultry industry. *E.coli* is a common inhabitant of the gastrointestinal tract of most animals, including birds, but not all *E.coli* isolates are capable of causing disease (1) . Avian pathogenic *E.coli* strains (APEC) cause several disease syndromes in farmed birds such as peritonitis (2), enteritis (3), airsacculitis, pericarditis, perihepatitis, salpingitis, synovitis, panophtalmitis (2-5) and swollen head syndrome (6) . The serological and bacteriological methods for typing microbes are often limited to a few select reference laboratories. Such typing precedures are not sensitive enough to further differentiate bacterial isolates. Bacterial isolates including *E.coli* can be differentiated into genetically distinct isolates using procedures that identify differences in the genetic composition of a microbial population. These techniques involve multilocus enzyme electrophoresis (MLEE) (7) , pulsed-field gel electrophoresis (PFGE) (8), random amplification of polymorphic DNA (RAPD) (9), bacterial restriction endonuclease digest analysis (BRENDA) (10), or restriction fragment length polymorphism (RFLP) analysis by either Southern blot or polymerase chain reaction (PCR) (10).

Most pathogenic *E.coli* avian isolates cannot be distinguished biochemically from the normal commensals inhabiting the gastrointestinal tract of birds. Diagnosis of *E.coli* infection currently relies on the phenotypic differentiation of pathogenic strains from nonpathogenic normal flora. Phenotypic differentiation methods can be time-consuming and complicated and are not routinely used in many clinical laboratories. Genotypic diagnosis may be accomplished by DNA colony blot hibridization to identify genes encoding virulence factors (11). Howewer, the use of radioactive isotopes and need for more time make this method unsuitable for many diagnostic laboratories.

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Various genotypic methods have been proven useful for species identification, epidemiological typing and determining the genetic relatedness among pathogenic and nonpathogenic bacteria (12, 13).

Genotyping of *E.coli* strains may aid in defining those that are specifically pathogenic for a certain host, and give guidance for epidemiological studies of sources of infection, and disease transmission. Using a molecular approach, arbitrary amplification of polymorphic DNA sequences, termed random amplification of polymorphic DNA (RAPD) analysis on arbitrarily primed PCR (AP-PCR) typing, (9,13) is one such new technique that is being used in many epidemiological studies. It is a fast, PCR based method of genetic typing based on genomic polymorphisms.

The purpose of the present study was to investigate the genetic differences in local *E.coli* isolates from colibacillosis suspicious hens.

Materials and Methods

Samples: In this study, a total of 110 intestinal organ samples, were collected from different broiler flocks. The samples were immediately transferred to laboratory where they were processed.

Culture: The samples were inoculated on sheep blood agar and MacConkey agar. Identification was based on biochemical tests, including hydrogen sulphide, citrate, urease and indole production (14). *E.coli* strains were stored in tryptone soy broth (Oxoid, Hamsphire, UK) with 15% glycerol at -70ºC.

RAPD analysis: A random COL-1 primer (3'-AAG AGC CCG T-5') (İontek, İstanbul, Türkiye) was used at a concentration of 1µM, according to the manufacturer's instructions (24).

DNA Extraction: Selected *E.coli* colonies were transferred into eppendorf tube containing 300µl distilled water. The tube was vortexed and incubated at 56° C for 30 min in boiling water bath. After suspension was added in 300 µl K buffer (20 mM Tris pH 8.0+ 150 mM NaCl + 10 mM EDTA+ %0.2 SDS) and 200 µg/ml proteinase K, incubate at 37º C for 2 h in boiling-water bath. Following incubation, mixture was boiled 30 min and vortexed. An equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11600 g for 10 min. The upper phase was transferred into a new eppendorf tube. Genomic DNA was precipitated with absolute ethanol and 0.3 M Na-Acetate at -20ºC for one hour or overnight. The pellet was washed twice with 300µl 90% and 70% ethanol and centrifuged at 11600 g for 5 min. The pellet was dried and suspensed in 50 µl distilled water and used target DNA in PCR.

Polymerase Chain Reaction (PCR) based on RAPD analysis: In RAPD analysis of *E.coli* strains, the reaction mixture was prepared in a total volume of 50µl consisting of 5µl template DNA, 10xPCR buffer (750 mM Tris HCl, 200 mM $(NH_4)SO_4$, 0.1 Tween 20), 3.5 mM $MgCl_2$, 200ul deoxynucleoside triphosphates, 1.25 U of Taq DNA polymerase (fermentas, Lithuania), and 1µM COL-1 primer (3'-AAG AGC CCGT-5'). The samples were amplified through 45 cycles at 30 s, 94ºC, 15 s, 36ºC, and 30s, 72ºC. In negative control reactions, the DNA template or the primer was replaced by sterile deionized water. RAPD products were visualized by means of ethidium bromide staining after electrophoresis in a 1,5% agarose gel with TBE buffer. Gel was photographed by polaroid gel cam and images were scanned and transferrred to computer for detailed analysis.

Results

E.coli was isolated from 48 (13,6%) of 110 samples. An example of the electrophoretic profiles generated by RAPD analysis using COL-1 primer of *E.coli* isolates from chickens is shown in Figure 1. Nine different band profiles were obtained.

Discussion

Infections with avian pathogenic *Escherichia coli* cause colibacillosis, an acute and mostly systemic disease resulting in significant economic losses in poultry industry worldwide. Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis being most typical. Environmental factors as well as the constitution of poultry or initial viral infections influence the outcome of avian pathogenic *Eschericia coli* infections (15).

The aim of this study was to investigate the genetic differences among *E.coli* isolates from chickens by RAPD. We used RAPD analysis to determine genetic heterogeneity of 48 *E.coli* strains isolated from chickens. The method was successfully applied to typing *E. coli* strains. RAPD recognized nine profiles among 48 isolates.

RAPD analysis uses oligonucleotide primers that amplify certain sections of the genome by PCR to produce identifiable banding patterns, which are useful in strain differentiation (16). The procedure has been widely used in a variety of bacteria (17-23).

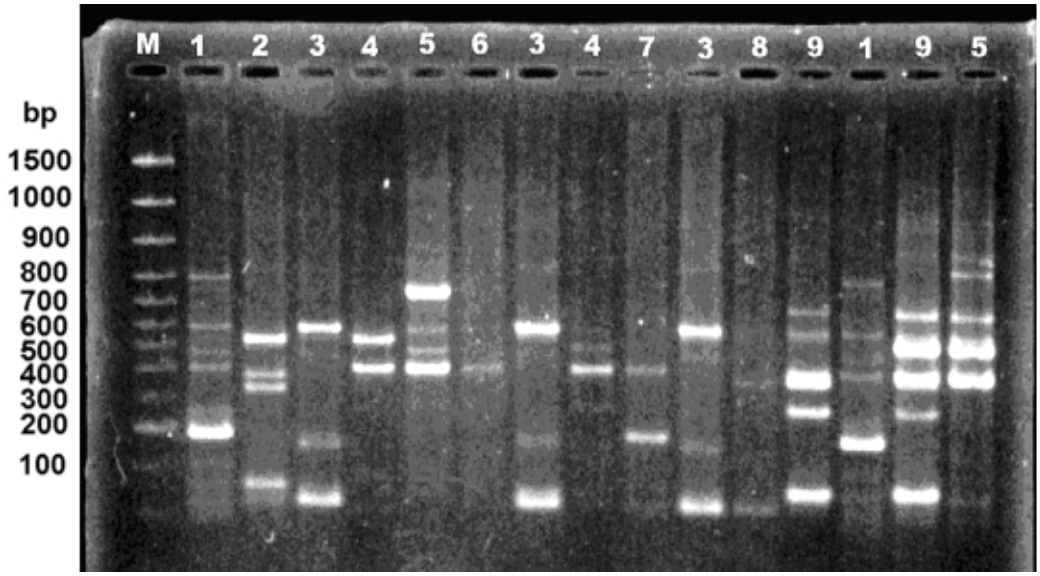

Figure1. RAPD-PCR profiles of *E.coli* strains from chickens. M: 1-kb ladder molecular size marker. Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 – profiles

In RAPD analysis, Maurer et al.(1998) found 16 different RAPD types in 84 % *E.coli* isolates (16). In this study, RAPD recognized nine different profiles among 48% *E.coli* isolates. In another study, Chansiripornchai et al.(2001) found 50 RAPD types by using two different primers in 55 *E.coli* strains (24). The use of different and more than one RAPD primers may improve differentiation power of RAPD process. Chansiripornchai et al. (2001) used six different primers in their research. The same researchers found from these primers that random primer number 4 gave highest discriminatory power on *E.coli* strain from different flock (24). For this reason random primer number 4 were preferred in this study. All the isolates were successfully typed using RAPD method. Cave et al. (1994) found 28 different patterns among 60 *E.coli* strains (25). These different patterns may be the result of exposing to different *E.coli* strains throughout the year.

The results of this study revealed that avian *E.coli* genetically very heterogenic. This result is in agreement with results of Chansiripornchai et al. (2001) and Maurer et al. (1998) finding (16,24). It is also not uncommon to find more than one *E.coli* genetic type from the same bird. Similar findings have been reported for *E.coli* in cattle (26), Staphylococcus aureus in dairy cows (27), and *E.coli* in poultry (28). Maurer et al (1998) encountered certain *E.coli* RAPD types throughout the year (16). The flocks do not have enough time to develop immunity to the *E.coli* genetic type due to the brief longevity of broiler and this may cause encountering different *E.coli* types.

The results of this study indicated that much genetic heterogeneity exists among *E.coli* isolates from chickens, and RAPD analysis have been proposed as alternative and used to characterize *E. coli* isolates from avian origin. The RAPD assay has been proved to have excellent discrimination ability due to the fact that the entire genome is the target in genotyping. Further genotyping studies must be done with strains originating from different sources to prove this implication.

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