

# Identification and Screening of Avian Pathogenic *E.coli* Virulence Factors in Palestine

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## Introduction:

*Escherichia coli* (*E. coli*) bacteria are common to many environments and exist in over 150 different strains. Avian pathogenic *E. coli* (APEC) strains cause diseases in birds at various ages. The introduction of such strains to the chicken via their respiratory tracts causes an invasive infection featured with multi-extraintestinal disorders, collectively known as Colibacillosis [1]. This causes massive death of chickens that leads to great economic losses for the poultry industry worldwide. Recent reports showed that the pathogenicity of APEC strains are associated with certain virulence genes that are located within the bacterial genome such as (*papC*, *astA*, *vat*, and *irp2*) and/or their *colV* plasmids such as (*tsh*, *iucD*, *iss*, and *cvi*) [2]. Identification and characterization of these genes are essential to implement an efficient disease control and prevention systems. The aim of this study is to screen genes that are associated with APEC diseases in Palestine.

## Materials and methods:

**Bacterial strains:** *E. coli* were isolated from poultry of 83 different farms in Palestine during Feb-Jun 2009. Sample collection was through the Central Veterinary laboratory - Ramallah – Palestine\*. All strains were isolated from visceral organs (liver, lung and heart) of poultry that had died from Colibacillosis with typical preceding symptoms like septicemia, respiratory infections and premature death. The (APEC O78 $\chi$ 7122. *astA*, *iss*, *papC*, *iucD*, *tsh*, *cvaC*; F.Dziva\* 2009) strain was used as positive controls to establish our multiplex PCR protocol.

**Culture and biochemical characterization:** Visceral organs were inoculated in *E.coli* Broth (EC Broth) at 44.5 °C for 18 to 24 h as pre-enrichment step, which will enrich *E.coli* growth and inhibit growth of other bacteria. Isolates were then cultured into 7% sheep blood agar, MacConkey agar and eosin-methylene blue agar. The identification of *E. coli* was based on the results of diagnostic tests, which included Gram stain, colony characterization, gas production and ability to be enriched in the EC Broth. Further molecular identification was performed by using specific designed *E.coli* primers for beta-D-glucuronidase gene (*uidA*).

**Antimicrobial sensitivity:** A sensitivity test for ten antimicrobial agents was carried out by the standard disk procedure on Muller Hinton agar. Ampicillin, Tetracycline, Amoxicillin, Neomycin, Gentamycin, Nitrofurantoin, Ciprofloxacin, Kanamycin, Chloramphenicol and Cephalixin standard paper disks were laid on the medium. The plates were incubated for 24 h at 37 °C and inhibition zones were measured.

**Multiplex PCR analyses:** Avian pathogenic *E. coli* isolates were analyzed by multiplex PCR protocol for the presence of the following genes: (*papC*, *astA*, *vat*, *irp2*, *tsh*, *iucD*, *iss*, and *cvi*). Primers were designed using PerlPrimr V1.0 software. Bacterial colony from overnight MacConkey agar at 37 °C was picked to be used as target for PCR amplification by boiling for 15 min. After centrifugation 2 ul of the supernatant was taken as template DNA. The amplification products were analyzed by gel electrophoresis on a 2.0 % agarose gel, stained with ethidium bromide, and photographed at UV exposure.

## Results:

Out of 83 isolates, 17 isolates had no ability to be enriched and characterized this could be related with misprocessing in organs inoculation process at pre-enrichment step. The other 66 isolates were identified as *E.coli* strains. The antibiotic sensitivity test for the 66 *E. coli* strains showed a high resistance level to six antimicrobial agents (Ampicillin: 81.82%, Tetracycline: 96.97%, Amoxicillin: 81.82%, Neomycin: 69.70%, Ciprofloxacin: 71.21% and Kanamycin: 78.79%). More than 95% of the isolates are resistance for three antimicrobial agents at least.

Nitrofurantoin and Cephalexin showed the lowest bacterial resistance with (18.18% and 12.12%) respectively. The multiplex PCR showed that *cvi* and *iss* genes have the highest rate of frequency with (100%), while *astA* presents in (98.48%) of isolates, *iucD* also has a high frequency rate with (78.79%). Whereas *vat*, *papC*, *irp2* and *tsh* genes present at (34.85%, 30.30%, 19.70%, 10.61%) of total isolates. This work is the first work that will establish virulence gene reference profile according APEC strains present in Palestine, and could a basic guideline for Colibacillosis epidemiology and controlling.

#### **References:**

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