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ORIGINAL ARTICLE

Phenotypic and genotypic analysis of pathogenic *Escherichia coli* virulence genes recovered from Riyadh, Saudi Arabia

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Abstract The current study was carried out to evaluate the phenotypic and genotypic characterization of avian pathogenic *Escherichia coli* recovered from Riyadh, Saudi Arabia. During the period of 10th February–30th May 2015, 70 *E. coli* strains were isolated from chicken farms located in Riyadh, Saudi Arabia. All strains were tested phenotypically by standard microbiological techniques, serotyped and the virulence genes of such strains were detected by polymerase chain reaction (PCR). Most of the recovered strains from chickens belonged to serotype O111:K58 25 strains (35.7%), followed by serotype O157:H7 13 strains (18.57%), followed by serotype O114:K90 10 strains (14.29%), then serotype O126:K71 9 strains (12.9%), serotype O78:K80 8 strains (11.43%) and in lower percentage serotype O114:K90 and O119:K69 5 strains (7.14%). The virulence genotyping of *E. coli* isolates recovered from broilers revealed the presence of the *uidA* gene in all the field isolates (6 serovars) examined in an incidence of 100%, as well as the *cvaC* gene was also present in all field isolates (6 serovars), while the *iutA* gene and the *iss* gene were detected in 5

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out of 6 field serovars in an incidence of 81.43% and 64.29%, respectively. Phenotypical examination of the other virulence factors revealed that 65 isolates were hemolytic (92.9%), as well as 15 isolates (21.42%) were positive for enterotoxin production. Meanwhile, 21 isolates (30%) were positive for verotoxin production, 58 isolates (82.86%) for the invasiveness and 31 isolates (44.29%) for Congo red binding activities of the examined serotypes.

1. Introduction

Strains isolated from localized and systemic disease processes in poultry are designed as avian pathogenic *Escherichia coli* (APEC), causing a number of diseases in domestic poultry, ultimately leading to disease and death, or a decrease in egg production or condemnation of carcasses. Among these diseases is a severe systemic form termed coliseptecemia, which is characterized by the presence of *E. coli* in the blood and colonization of the organism in organs including the heart, liver and spleen (Barnes et al., 2003).

The disease inducing potential of these isolates has been explained by the occurrence of specific virulence factors. Many virulence factors have been associated with avian pathogenic *E. coli* (APEC) strains, although their role in the pathogenesis is not well known (Mellata et al., 2003). Despite extensive literature on virulence factor *E. coli* bacteria, unambiguous markers of virulence have not been identified yet. The relationship between serotyping and virulence is not straightforward either and raises the question whether *E. coli* infections of poultry should mainly be considered as opportunistic (Landman and Cornelissen, 2006). The main goal of this study was to investigate the characteristics of APEC in the boiler chickens collected from different farms located in Riyadh, Saudi Arabia.

2. Materials and methods

2.1. Samples

During the period of 10th February–30th May 2015, samples from the blood and different organs (liver, yolk sac, spleen, ovary and joint) were aseptically collected from 110 broiler chickens and showed signs of colisepticemia in poultry farms, located in Riyadh, Saudi Arabia. Samples were transferred to the laboratory in an ice box to be cultured immediately.

2.2. Isolation and identification of E. coli

Samples were primarily inoculated in pre-enrichment media then streaked on MacConkey agar medium and incubated aerobically at 37 °C. After an overnight incubation, a part of single typical well isolated lactose fermenting colony was tested for sorbitol fermentation by culturing on sorbitol MacConkey agar and sorbitol phenol red agar media, then incubated at 37 °C overnight. Morphological, cultural and biochemical examinations were carried out according to Murray et al. (2003). Isolates that were primarily identified by biochemical tests were then subjected to serological identification using diagnostic polyvalent and monovalent *E. coli* antisera (Welcome diagnostic antisera). Diagnostic *E. coli* O157 antisera (Difco) and H7 anti-sera (Difco) were used for serological identification of *E. coli* O157:H7.

2.3. Detection of virulence factors by polymerase chain reaction (PCR)

Extraction of DNA of each culture by the boiling method was performed according to Croci et al. (2004) PCR design and amplification conditions were performed using PCR primer pairs with reference to published sequence data for the *uidA* gene of *E. coli* (Croci et al., 2004) encoding b-glucuronidase specific for *E. coli* (Heininger et al., 1999), and increase in serum survival of iss gene of *E. coli* (Yaguchi et al., 2007), aerobactin *iutA* of *E. coli* (Delicato et al., 2003), and the *cvaC* gene (Rocha et al., 2008). Details of the nucleotide sequence, the specific gene region amplified, and the size of the PCR product for each primer pair are listed in Table 1. PCR products were visualized by agarose gel electrophoresis according to Sambrook et al. (1989).

2.4. Phenotypic detection of virulence factors of E. coli isolates

Hemolytic activity (hemolysin) was tested using 5% defibrinated sheep blood agar. The ability to produce heat stable

Table 1 Primer sequences used for PCR amplification.

Target gene	Sequence (5–3)	Amplicon size bp
uidA gene of *E. coli*	F(ATC ACC GTG GTGACG CATGTCGC) R(CAC CAC GAT GCC ATG TTC ATC TGC)	468
Iron uptake transport gene *iutA*	F(GGC TGG ACA TGG GAA CTG G) R(CGT CGG GAA CGG GTA GAA TCG)	300
Colicin v *cvaC*	F(CAC ACA CAA ACG GGA GCT GTT) R(CTT CCC GCA GCA TAG TTC CAT)	680
Increased serum survival gene *ISS*	F(ATG TTA TTT TCT GCC GCT CTG) R(CTA TTG TGA GCA ATA TAC CC)	266