Determination of Adhesin Encoding Genes in Escherichia coli Isolates from Omphalitis of Chicks

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Abstract: Problem statement: Omphalitis is one of the most common causes of mortality in chicks during the first week after hatching. Escherichia coli strains are the most common isolated bacteria from omphalitis cases of chickens. Bacterial colonization in the host cells surfaces is a critical first step in the pathogenesis of avian pathogenic Escherichia coli isolates. Thus the current study was undertaken to determine the presence and prevalence of several adhesin-encoding genes in E. coli isolates from omphalitis of chicks. Approach: One hundred four E. coli isolates were recovered from omphalitis cases and were identified by standard biochemical tests. The omphalitis-derived isolates were examined for the presence of fimbrial and non-fimbrial adhesin-encoding genes by PCR technique. Results: Most (93.26%) of the E. coli isolates exhibited at least one of the examined adhesin-encoding genes. None of the isolates contained the afa D-B-C, afa E-VIII and f17A genes. The two most prevalent genes were crl (87.50%) and fimH (77.88%). P (papC) and S (sfa) fimbriae encoding genes were detected in 8 (7.69%) and 5 (4.80%) isolates respectively. Seven combination patterns of the adhesin-encoding genes were detected. In 83 (79.80%) isolates combinations of 2-4 genes were detected. The gene combinations of crl-fimH and fimH-papC were the two most prevalent patterns respectively. Fourteen (13.46%) isolates showed crl gene alone and 7 (6.73%) isolates were negative for examined genes. Conclusion: The current study showed that some of the adhesin-encoding genes are more prevalent in E. coli isolates from omphalitis of chicks but, E. coli isolates may be expressing still unknown adhesins that could have a role in the pathogenicity of omphalitis-derived isolates.

Key words: E. coli, adhesin genes, omphalitis, virulence factor, chicken

INTRODUCTION

Omphalitis is infectious and non-contagous condition of yolk sac which accompanied by unhealed navels in young fowl. The affected chicks appear normal until a few hours before death (Kahn et al., 2008). Bacterial infection of navel area is one of the most common causes of mortality in chicks during the first week after hatching (Pattison et al., 2008). Several bacteria such as Proteus spp., Enterobacter spp., Pseudomonas spp., Klebsiella spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Bacillus cereus and Enterococcus have been isolated from yolk sac infection of birds (Cortes et al., 2004). Escherichia coli (E. coli) is the most common contaminant of yolk sacs in chickens and about 70% of chicks with omphalitis had this bacterium in their yolk sacs. On the other hand, it is common to recover low numbers of E. coli from normal yolk sacs (Saif et al., 2008). For many years, it was believed that E. coli isolates from omphalitis cases were avirulent or of low virulence. E. coli is one of the opportunistic pathogens responsible for a number of disease conditions such as yolk sac infection, air sac disease, pericarditis, enteritis, omphalitis, coligranuloma, colibacillosis (Ahmad et al., 2009). However in genotypic studies omphalitis isolates tended to be more similar to commensal isolates than Avian Pathogenic E. coli (APEC) isolates (Amabile de Campos et al., 2005). The role of virulence factors in pathogenesis of APEC isolates have not been fully elucidated but considerable progress has been made recently to establish the mechanisms of pathogenesis (Stehling et al., 2007).
Bacterial colonization in the epithelial surfaces is considered a critical first step in the pathogenesis of APEC isolates (Ramirez et al., 2009a). Interaction between bacteria and host tissue, or soluble protein, is necessary for pathogenesis, which occurs through primary adhesion, invasion into the host and tissue-specific colonization (Ramirez et al., 2009b). F1 fimbriae are expressed by E. coli in the respiratory tract, lungs and air sacs of infected birds, indicating a possible role during initial stages of disease whereas P-fimbriae may play a role in later stages of infection (Edelman et al., 2003; Pourbakhsh et al., 1997). S-fimbriae may promote adherence of bacteria to intestinal epithelial and tracheal cells. Afimbrial adhesions encoding genes have been detected in E. coli isolates associated with diarrhea and septicaemia in calves and piglets (Lymberopoulos et al., 2006; McPeake et al., 2005). The role of curli fimbriae (encoded by crl and csgA genes) in pathogenesis of E. coli isolates remains unstudied, although may be mediates E. coli adherence to fibronectin and laminin (Ghanbarpour et al., 2010; La Ragione et al., 2000).

In Iran mortality and morbidity rates of yolk sac infection in broiler chickens were reported 10% and 5-10% respectively (Kalidari et al., 2009). The purpose of this study was to determine the presence and prevalence of several adhesion-encoding genes in E. coli isolates from omphalitis of chicks.

**MATERIALS AND METHODS**

In the period of April 2007 to December 2008, 104 E. coli isolates were recovered from omphalitis cases of broilers from 18 different flocks. Isolation and biochemical identification of E. coli specifically was targeted in the specimens. Standard biochemical tests and bacteriological methods were used to confirm the E. coli strains. Isolates were stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30% sterile glycerol.

Five E. coli strains were used as positive controls: 28C (papC+); 1404 (f17A+); 239KH89 (afa E-VIII); J96 (sfa+). Laboratory nonpathogenic E. coli strain MG1655 was used as a negative control. All the reference strains were from the bacterial collection of Microbiology Department of Ecology National Veterinary Toulouse, France.

All E. coli isolates and reference strains were harvested from an overnight Luria-Bertani broth culture to prepare DNA extract by boiling. Three hundred micro-litres was centrifuged for 30 sec and re-suspended in 50 µL of sterile water, boiled for 10 min and re-centrifuged for 30 sec, 2 µL of the supernatant was added to the reaction mixtures. The PCR assays were performed in a total volume of 50 µL.

The isolates were examined by PCR assay for the presence of the genes encoding Afa E-8 adhesion described by Lalioui et al. (1999), for fimH, papC and afaI (B-C) encoding operons by Johnson and Stell (2000), for F17 family genes by Van Bost et al. (2003), for curli fimbriae encoding gene (crl) by Maurer et al. (1998) and Sfa/focD-E encoding operon by Yamamoto et al. (1995). The specific primers (TAG Copenhagen, Denmark) used for amplification of the examined genes and expected size of products are presented in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′-3′)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>afaI B-C</td>
<td>GCTGGGCAGCAAACCTGATAAACTCTC</td>
<td>750</td>
<td>Johnson and Stell (2000)</td>
</tr>
<tr>
<td>afa E-8</td>
<td>CTAACCTGCGAGCAGCTGTTT</td>
<td>302</td>
<td>Lalioui et al. (1999)</td>
</tr>
<tr>
<td>crl</td>
<td>TTTCAGATTCAGCGTCGTC</td>
<td>250</td>
<td>Mauret et al. (1998)</td>
</tr>
<tr>
<td>f17A</td>
<td>GCAGAAAAATTCAGATTGCTTTGGG</td>
<td>537</td>
<td>Van Bost et al. (2003)</td>
</tr>
<tr>
<td>fimH</td>
<td>TGCAGAAAGGATAAGCCGTTTAGTGAATATAC</td>
<td>508</td>
<td>Johnson and Stell (2000)</td>
</tr>
<tr>
<td>papC</td>
<td>GTGGCGGATGAAATGTCGGGATGCAAATTATCAAAACCTGGCCA</td>
<td>205</td>
<td>Johnson and Stell (2000)</td>
</tr>
<tr>
<td>sfa/focD-E</td>
<td>CGGAGGAGAATTACAAACCTGGCCAATCTCTCTTATCAAACTGGCCA</td>
<td>410</td>
<td>Yamamoto et al. (1995)</td>
</tr>
</tbody>
</table>

RESULTS

PCR assays revealed that 97 (93.26%) E. coli isolates exhibited at least one of the examined fimbrial and non-fimbrial adhesin-encoding genes. None of the isolates contained the afaI B-C, afa E-VIII and f17A genes. All of the detected adhesin genes were present alone or in combination with each others.
Out of 104 examined *E. coli* isolates 91 (87.50%) were positive for *crl* gene which was the most prevalent genetic marker. Among 91 *crl* positive isolates, 14 (15.38%) isolates exhibited the gene alone and in 77 (84.61%) isolates were in combination with F1, S and P fimbriae encoding genes (Table 2).

The genetic marker for F1 fimbriae was found in 81 (77.88%) isolates, which was the second most prevalent adhesion gene. All of the *fimH* positive isolates had one of the other examined genes.

Eight (7.69%) isolates were positive for P fimbriae encoding gene whereas *sfa/focD-E* gene was detected in five (4.80%) isolates in combination with *fimH*, *papC* and *crl* gene sequences.

Analyses of PCR results for determination of adhesion genes showed that the examined genes existing in several patterns of gene combination (Table 2). In 83 (79.80%) isolates combinations of 2-4 genes were detected. The gene combination of *crl-fimH* was the most prevalent (69.23%) pattern followed by *fimH-papC* (3.84%).

### DISCUSSION

According to faulty management at the hatchery and breeding farms the sources of the omphalitis-derived bacteria were variable including fecal contamination of eggshell, poor hatchery hygiene, poor quality control measures, contaminated chick boxes or supply contaminated vehicles and contaminated feeding of day old chick in improper disinfection of farms after previous flock (Gordon and Jordon, 1982; Iqbal et al., 2006; Munir et al., 2004). Coliform and *E. coli* densities remain fairly consistent in poultry litter whereas *E. coli* can penetrate the shell and causes decrease in hatchability (Sander et al., 2003). Omphalitis-derived isolates frequently are not included in APEC group because some authors have mentioned that these *E. coli* isolates are just opportunistic and non pathogenic agents (Rosario et al., 2005).

The present study, among 104 *E. coli* isolates from omphalitis cases 93.26% were positive for one of the examined genes. These isolates were positive for one of the *crl, fimH, papC* and *sfa/foc* genes. Similarly, Knobl et al. (2004) detected the fimbrial and afimbrial adhesins in omphalitis-derived *E. coli* isolates encoded by *fim* (type 1 or F1 fimbriae), *pap* (P fimbriae), *sfa* (S fimbriae) and *afa* (afimbrial adhesion) operons. The principle adhesions described in APEC isolates are type 1 (F1), P and curli fimbriae (Saif et al., 2008). In this study, 87.50% of the isolates possessed the *crl* gene. McPeake et al. (2005) and Delicato et al. (2003) reported that 100% of APEC isolates were positive for curli encoding genes whereas Amabile de Campos et al. (2005) indicated that 16.7 % of *E. coli* isolates from colisepticemic cases were positive for *crl* gene. In the present study, 77.88% of isolates were positive for *fimH* gene in combination with other detected genes. F1 fimbrina encoding gene (*fim*) was detected in 96% of *E. coli* isolates from omphalitis cases by colony hybridization test (Knobl et al., 2004). In several studies *fimH* gene was detected in 100% of *E. coli* isolates from colisepticemic poultry (Moulin-Schouleur et al., 2007; Vandekerchove et al., 2005). However this gene was detected in 96.5 and 92% of avian pathogenic and fecal *E. coli* isolates respectively (Delicato et al., 2003). The *fimC* gene of fim operon was found in 90 and 92% of APEC isolates (Kawano et al., 2006; Ewers et al., 2004). Arne et al. (2000) reported that, although FimH is required for adhesion to cultured chicken tracheal or pharyngeal cells, lack of *FimH* favors in vivo colonization of the trachea of chickens. P fimbriae encoding genes *papC* and *pap-E* have been detected in *E. coli* isolates from different lesions of chickens. In the present study 7.69% of the examined isolates were positive for *papC* gene. Knobl et al. (2004) found that 8% of *E. coli* isolates from omphalitis-derived and

### Table 2: Adhesin genes and their combination patterns detected in 104 *E. coli* isolates from omphalitis

<table>
<thead>
<tr>
<th>Combination patterns</th>
<th>crl</th>
<th>fimH</th>
<th>papC</th>
<th>sfa/foc</th>
<th>afaIB-C</th>
<th>afaE-8</th>
<th>f17A</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>crl-fimH-papC-sfa/foc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (0.96)</td>
</tr>
<tr>
<td>crl-fimH-sfa/foc</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (1.92)</td>
</tr>
<tr>
<td>crl-papC</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72 (69.23)</td>
</tr>
<tr>
<td>fimH-papC</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (1.92)</td>
</tr>
<tr>
<td>fimH-sfa/foc</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 (3.84)</td>
</tr>
<tr>
<td>papC-sfa/foc</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (0.96)</td>
</tr>
<tr>
<td>crl</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (0.96)</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7 (6.73)</td>
</tr>
<tr>
<td>Total No. and (%)</td>
<td>91 (87.50)</td>
<td>81 (77.88)</td>
<td>8 (7.69)</td>
<td>5 (4.80)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>104 (100)</td>
</tr>
</tbody>
</table>
salpingitis were positive for pap operon. McPeake et al. (2005) found that 41.2% of isolates from septicaemic birds possessed P-fimbriae (pap) gene sequences, compared with only 15.6% from E. coli isolated from healthy birds. Delicato et al. (2003) detected the pap operon genes papA, papG and felA in less than 20% of the isolates, their frequency still was significantly greater in colibacillosis isolates than in fecal ones. A study on avian pathogenic E. coli isolates indicated that pap gene were significantly associated with septicaemic and swollen head syndrome strains; but were not associated with omphalitis isolates (Amabile de Campos et al., 2005). The pap gene sequences have been detected in a high frequency in APEC strains (Ngeleka et al., 2002; Rodriguez-Siek et al., 2005; Stordeur et al., 2002). In different studies papC gene were detected in 40.4, 35.7 and 22.7% of APEC isolates (Ewers et al., 2004; Kawano et al., 2006; Rodriguez-Siek et al., 2005). In the current study sfa/foc gene was detected in 4.80% of E. coli isolates from omphalitis cases, whereas none of the isolates were positive for afal B-C, afa E-8 and f17A sequences. In Brazil, sfa genes were detected with a higher frequency in E. coli isolates from omphalitis (16%) than in strains from salpingitis and chronic respiratory disease (Knobl et al., 2004). Amabile de Campos et al. (2005) reported that only few septicaemic strains present afa and sfa adhesion sequences (12.5 and 4.16%, respectively). A study on APEC isolates showed a low frequency of afa (5.5%) and sfa (4.4%) adhesion sequences (Stordeur et al., 2002). In a few E. coli isolates from avian cellulitis sfa and f17A genes were detected, whereas the examined isolates were negative for afal B-C and afa E-8 adhesion sequences (Ghanbarpour et al., 2010). Although F17 fimbriae and the Afa adhesions occur on less than 10% of APEC isolates, there are evidence that E. coli isolates expressing afa-8 gene cluster are lethal for 1-day-old chikens and are able to reproduce clinical sings and lesions of colibacillosis (Ewers et al., 2007; Stordeur et al., 2004). In the present study, 79.80% of isolates showed seven combinations of 2-4 operons, which combination of crl-fimH were the most prevalent patterns. In omphalitis-derived E. coli strains two combinations of adhesion encoding gene fim-pap and fim-sfa were reported previously (Knobl et al., 2006). Most of cellulitis, septicaemic and swollen head syndrome isolates of E. coli showed two and three to four adhesion-related DNA sequences (Amabile de Campos et al., 2005; Ghanbarpour et al., 2010; McPeake et al., 2005; Ngeleka et al., 1996).

Rosario et al. (2005) have defined some characteristics of virulent E. coli strains associated with chick omphalitis and suggested the existence of a limited number of clone complexes that possess particular traits that make the isolates able to cause disease in poultry. Several studies have shown that omphalitis-derived isolates E. coli isolates produce colicin and also were positive for ipaH gene, which could play a role in the pathogenicity of bacteria (Blanco et al., 1997; Cortes et al., 2004).

CONCLUSION

The current study showed that some of the adhesin encoding genes are prevalent in Escherichia coli isolates from omphalitis of chicks. Presence of DNA sequences, related to fimbrial expression does not mean that particular fimbria is expressed. On the other hand, E. coli isolates may be expressing still unknown adhesions that could have a role in the pathogenicity of omphalitis-derived isolates. Therefore, other studies (such as experimental and molecular examinations) should be carried out to establish the importance of fimbrial and non-fimbrial adhesion genes and their expression in the pathogenesis of omphalitis associated isolates.

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REFERENCES


