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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 *afaI 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-contagious 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 opportunist pathogen responsible for number of disease conditions such as yolk sac infection, air sac disease, perihepatitis, 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).

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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-8+*); J96 (*sfa+*, *fimH+*, *crl+*), A30 (*afaI B-C+*). 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* adhesin 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.

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.

Table 1: Primers used for PCR amplifications

Gene	Primer sequence (5′-3′)	Product size (bp)	Reference
afaI B-C	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	Johnson and Stell (2000)
afa E-8	CTAACTTGCCATGCTGTGACAGTA TTATCCCCTGCGTAGTTGTGAATC	302	Lalioui *et al*. (1999)
crl	TTTCGATTGTCTGGCTGTATG CTTCAGATTCAGCGTCGTC	250	Maurer *et al*. (1998)
f17A	GCAGAAAATTCAATTTATCCTTGG CTGATAAGCGATGGTGTAATTAAC	537	Van Bost *et al*. (2003)
fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	Johnson and Stell (2000)
papC	GTGGCAGTATGAGTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA	205	Johnson and Stell (2000)
sfa/focD-E	CGGAGGAGTAATTACAAACCTGGCA CTCCGGAGAACTGGGTGCATCTTAC	410	Yamamoto *et al*. (1995)

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

Gene --------------------------- Combination patterns	*crl*	*fimH*	*papC*	*sfa/foc*	*afaIB-C*	*afaE-8*	*f17A*	Total No. (%)
crl fimH papC sfa/foc	+	+	+	+	-	-	-	1 (0.96)
crl fimH sfa/foc	+	+	-	+	-	-	-	2 (1.92)
crl- fimH	+	+	-	-	-	-	-	72 (69.23)
crl- papC	+	-	+	-	-	-	-	2 (1.92)
fimH-papC	-	+	+	-	-	-	-	4 (3.84)
fimH-sfa/foc	-	+		+	-	-	-	1 (0.96)
papC-sfa/foc	-	-	+	+	-	-	-	1 (0.96)
crl	+	-	-	-	-	-	-	14 (13.46)
Negative	-	-	-	-	-	-	-	7 (6.73)
Total No. and (%)	91 (87.50)	81 (77.88)	8 (7.69)	5 (4.80)	-	-	-	104 (100)

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*, *pap* 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). It has been shown that *E. coli* isolates from breeder farm; hatchery and broiler farms carried the virulence associated genes (Dias da Silveira *et al*., 2002). In 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 adhesin) 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 fimbria 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 *papE-F* have been detected in *E. coli* isolates from different lesions of chickens. In the present study 7.69% of the examined isolates were positive e for *papC* gene. Knobl *et al*. (2004) found that 8% of *E. coli* isolates from omphalitis-derived and salpangitis 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 *afaI 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* adhesin 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 *afaI B-C* and *afa E-8* adhesin 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 collibacillosis (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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