

OCCURRENCE OF ADHESIN-ENCODING OPERONS IN *ESCHERICHIA COLI* ISOLATED FROM BREEDERS WITH SALPINGITIS AND CHICKS WITH OMPHALITIS

Terezinha Knöbl¹; Tânia A. Tardelli Gomes²; Mônica A. Midolli Vieira²; José A. Bottino³;
Antonio J. Piantino Ferreira^{3*}

¹Faculdade de Medicina Veterinária, Faculdades Metropolitanas Unidas, São Paulo, SP, Brasil; ²Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil; ³Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brasil

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ABSTRACT

The occurrence of *fim*, *pap* and *sfa* operons in *Escherichia coli* isolated from breeders with salpingitis and chicks with omphalitis was evaluated. Analysis of 100 *E. coli* isolates, by colony hybridization tests, showed that 78 (78%) were *fim*⁺, one (1%) was *sfa*⁺, seven (7%) were *fim*⁺ associated with *pap*⁺, eight (8%) were *fim*⁺ and *sfa*⁺, one (1%) was *fim*⁺*pap*⁺*sfa*⁺ and five (5%) isolates did not hybridize with any probe. These results suggest that *fim* adhesion-encoding operon plays an important role in pathogenesis of *E. coli* infection in chickens with salpingitis and omphalitis.

Key words: *Escherichia coli*, adhesion-encoding operon, salpingitis, omphalitis, poultry

INTRODUCTION

Pathogenic *Escherichia coli* can be isolated from a variety of well-defined conditions that occur in poultry: airsacculitis, chronic respiratory disease (CRD), salpingitis, omphalitis, peritonitis, swollen head syndrome (SHS), colisepticemia, synovitis, cellulitis and coligranuloma (2,21). The agent is regarded as one of the principal causes of morbidity and mortality in poultry and is a leading cause of worldwide economic losses in the poultry industry, including that of Brazil.

Salpingitis is characterized by a subacute suppurative process compromising the oviduct, embryo development and day-old chick livability. In adult female birds, *E. coli* can infect the reproductive tract by resulting in salpingitis, oophoritis and peritonitis inducing chicken to death. Early infection in breeders may be clinically asymptomatic; occasionally it can be responsible for ensuing increase in embryonic mortality in the hatchery (2,21).

Omphalitis, generally, is an infection that affects chicks during the hatching phase. Disease is an inflammation of the

umbilicus and affected birds are involved in culling in the first week of age and death. Usually, fecal contamination of eggs or salpingitis is considered the most important source of infection. Both infections are close related and the virulence factors remain unclear (2,21).

The ability of bacteria to adhere to host epithelial cells is considered to be a prerequisite for the establishment of infectious diseases, mainly through expression of fimbriae (3,22,24). Avian pathogenic *E. coli* (APEC) generally possess type 1 and P fimbriae (12,16,26,29).

Type 1 fimbriae are characterized by the ability to agglutinate chicken and guinea pig erythrocytes in the absence of D-mannose. They consist of a major protein, FimA, associated with ancillary proteins, FimF, FimG and the adhesin FimH, encoded by the *fim* gene cluster (18,25). This type of fimbria is common among Enterobacteriaceae and several variants have been associated with APEC (4,6,28,36). Their role in infection is unclear, although it has been suggested that they may be involved in the initial stages of colonization of upper respiratory tract (6,7,11,16,31).

*Corresponding Author. Mailing address: Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando M. Paiva, 87, Cidade Universitária. 05508-900, São Paulo, SP, Brasil. E-mail: af.piantino@fmvz.usp.br

P fimbriae are associated with pyelonephritis in humans and are encoded by the *pap* operon (15,23). The role of P adhesins in APEC has not yet been fully elucidated, but they appear to be involved in the colonization of internal organs (26,30).

The S fimbriae are mannose-resistant adhesins (MRHA), encoded by the *sfa* operon, that recognizes α -sialyl- β -2,3-Galactose receptors, present on human and calf erythrocytes (8,12). The presence of S fimbriae correlates with pathogenicity of *E. coli* in human meningitis and septicemia (8,12,29). The role of S fimbriae in the pathogenicity of APEC remains unclear.

The purpose of this survey was to compare the occurrence of *fim*, *pap* and *sfa* genes in *Escherichia coli* isolated from breeders with salpingitis and from chicks with omphalitis.

MATERIALS AND METHODS

Bacterial strains and growth conditions

A total 100 isolates of *E. coli* were isolated from poultry in the São Paulo State of Brazil. The isolates were obtained from: (i) oviducts of broiler breeders with salpingitis (n=50) and (ii) yolk sacs of day-old chicks with omphalitis (n=50); Standard bacteriological methods were employed for isolation and identification of the organisms (2). All isolates were stored at -20°C in Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) to which 15% glycerol was added after incubation.

Colony hybridization

The test isolates were examined by colony blot hybridization (20), using specific DNA probes labeled with [α -d-³²P]-dATP by nick translation (20).

The DNA probe used to detect *fim* B-H genes was a 9.6 Kb *Hind*III-*Sal*I fragment from recombinant plasmid, pIB254 (17). Detection of *pap* and *sfa* genes involved oligonucleotide fragments obtained by the polymerase chain reaction, as described by Le Bouguenec and coworkers (19). The prototype wild-type strains from which DNA probes are derived were used as positive controls of each probe. *E. coli* K12 C600/pBR322 was used as negative control in all hybridization tests.

Statistical methods

Differences between groups of isolates were tested by using the software "SPSS for Windows" version 9.0 (SPSS, Chicago, IL, USA), by Fisher's exact test, using X² of Pearson, for low-value data. Differences were considered to be significant when $p < 0.05$.

RESULTS

Of the 100 *E. coli* isolates examined in both diseases, 78 (78%) isolates hybridized with *fim* operon. Considering that 50 isolates were obtained from salpingitis and 50 from

omphalitis, 43 (86%) and 35 (70%) hybridized with the probe for *fim*B-H detection, respectively. There were no significant differences between both groups in relation to the presence of *fim* gene ($P < 0.05$).

sfa operon was detected only in one (2%) isolate from omphalitis. The association of *fim* and *pap* genes were detected in three (6%) from salpingitis and four (8%) from omphalitis. When the association between *fim* and *sfa* was considered, only one (2%) and seven (14%) hybridized with these probes in salpingitis and omphalitis, respectively. There were significant differences regarding the presence of these operons in *E. coli* isolates from breeders with salpingitis and chicks with omphalitis ($P < 0.05$). Detection of combination of three operons was observed only in one (2%) isolate from salpingitis. Two (4%) isolates from salpingitis and three (6%) from omphalitis groups did not hybridize with any probe employed. The comparisons of all results are shown in the Table 1.

Table 1. Distribution of *fim*, *pap* and *sfa*-related nucleotide sequences among avian *Escherichia coli* isolates.

Genotype pattern	Number of probe-positive isolates (%) from		
	Salpingitis	Omphalitis	Total
<i>fim</i> ⁺	43 (86)	35 (70)	78 (78)
<i>sfa</i> ⁺	0 (0)	1 (2)	1 (1)
<i>fim</i> ⁺ <i>pap</i> ⁺	3 (6)	4 (8)	7 (7)
<i>fim</i> ⁺ <i>sfa</i> ⁺	1 (2)	7 (14)	8 (8)
<i>fim</i> ⁺ <i>pap</i> ⁺ <i>sfa</i> ⁺	1 (2)	0 (0)	1 (1)
None*	2 (4)	3 (6)	5 (5)
Total	50	50	100

*None of these isolates hybridized with *fim*, *pap* or *sfa* operon probe.

DISCUSSION

Certain genotypic traits associated with *E. coli* causing extraintestinal disease in humans are frequently found in APEC. Epidemiological investigations have shown a good correlation between the occurrence of certain human diseases and the presence of specific virulence factors in *E. coli* (1,5,33). Operons encoding P, S and Afa adhesins, production of α -haemolysin and cytotoxic necrotizing factor type 1 (CNF1) contribute to the pathophysiology of urinary tract infections, while genes encoding for S fimbriae, K1 capsule and Ibe 10 protein are correlated with the pathogenesis of neonatal meningitis (13,14).

APEC can be responsible for many localized and systemic diseases in poultry, but the pathophysiology of these infections remains unclear. In the present study it has been shown that

the presence of *pap* and *sfa* operons varies among strains associated with different diseases. Table 1 shows, also, that *pap*, *sfa* or any operon combination were detected with a lower frequency in salpingitis and omphalitis. By the other hand, *fim* operon was detected with a higher frequency in two groups, 70% from the omphalitis and 86% from the salpingitis isolates.

This study also confirms previous observations that mannose-sensitive adhesins are frequently present in APEC and type 1 fimbriae may play a role in tracheal colonization (6,9,10,11,31,32,34,35). Pourbakhsh and coworkers (26) investigated the site of *in vivo* expression of type 1 and P fimbriae in experimentally inoculated chickens and suggested that type 1 fimbriae are involved in the initial stages of bacterial colonization of the upper respiratory tract, whereas P fimbriae may be involved in the colonization of internal organs and in the development of septicemia, although this fimbria was not detected with a high frequency in this survey. There is no enough information in the literature to establish a correlation between the presence of type 1 fimbriae in APEC with penetration of mucus and ascending contamination of the vagina, following cloacal colonization in breeder chickens. Nevertheless, such association is likely.

The S fimbriae are able to promote the adherence of *E. coli* to endothelial and epithelial cells of the coroid plexus and cerebral ventriculus in man (27). This type of fimbria was rarely detected in APEC and its role in the pathogenesis of avian colibacillosis is presently unclear. It is possible that bacterium with S fimbriae is from human origin and that chicks become infected due to poor hygiene during handling of eggs in the hatchery. Experimental infection will be useful for gaining an understanding the role of fimbrial expression and the mechanisms of adhesion to specific tissues.

A continuous surveillance study should be introduced to assess the importance of type 1 fimbriae in the pathogenesis of avian colibacillosis regarding salpingitis and omphalitis infection for to implement an immunization program.

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RESUMO

Ocorrência de operons codificadores de adesinas em *Escherichia coli* isolada de aves reprodutoras com salpingite e de pintinhos com onfalite

Ocorrência dos operons *fim*, *pap* e *sfa* em amostras de *Escherichia coli* isoladas de reprodutoras com salpingite e pintinhos com onfalite foi avaliada. A análise de 100 amostras

através dos testes de hibridização de colônia mostrou que 78 (78%) amostras eram *fim*⁺, uma (1%) era *sfa*⁺, sete (7%) eram *fim*⁺ associada a *pap*⁺, oito (8%) eram *fim*⁺ e uma (1%) era *fim*⁺*pap*⁺*sfa*⁺ e cinco (5%) amostras não hibridizaram com nenhuma sonda. Estes resultados sugerem que o operon *fim* pode ter um importante papel na patogenia da infecção de *Escherichia coli* em reprodutoras com salpingite e pintinhos com onfalite.

Palavras-chave: *Escherichia coli*, operon codificador de adesina, genes, salpingite, onfalite, aves

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