Detection of *pap*, *sfa*, *afa* and *fim* Adhesin-Encoding Operons in Avian Pathogenic *Escherichia coli*

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ABSTRACT

The occurrence of *fim*, *pap*, *sfa*, and *afa* genes was evaluated in 200 *Escherichia coli* strains isolated from chickens diagnosed with omphalitis, salpingitis, swollen head syndrome, or chronic respiratory disease. Analysis of the strains by colony hybridization tests demonstrated that 96% of the isolates were *fim*$^+$, 16% were *pap*$^+$, and 6% were *sfa*$^+$. None of the isolates was *afa*$^+$. The *fim* gene occurred in strains from all diseases evaluated, with no significant differences among the isolates. Conversely, significant differences were observed in relation to *pap* and *sfa* genes. Of the strains tested, 8% from either salpingitis or omphalitis were positive for *pap* gene, compared with 28% from swollen head syndrome and 20% from chronic respiratory disease. Evaluation of the *sfa* gene indicated its presence in 4% of the salpingitis and chronic respiratory disease isolates and 6% of omphalitis, but *sfa* was not observed in isolates from swollen head syndrome. An in vitro adherence test showed that 97% of the isolates were capable of adhering to tracheal epithelium.

INTRODUCTION

Avian pathogenic *Escherichia coli* can be isolated from a variety of well-defined conditions that occur in poultry, including airsacculitis, chronic respiratory disease, salpingitis, omphalitis, peritonitis, swollen head syndrome, colisepticemia, synovitis, cellulitis, and coligranuloma.[1] 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 from disease in the poultry industry.

The ability of bacteria to adhere to host epithelial cells is considered a prerequisite for the establishment of infectious diseases, mainly through expression of fimbriae.[2–4] Avian pathogenic *E. coli* generally possess type 1 and P fimbriae.[5–8]

Type 1 fimbriae are characterized as having 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, encod-

ed by the *fim* gene cluster.[9,10] This type of fimbria is common among Enterobacteriaceae, and several variants have been associated with avian pathogenic *E. coli*.[11–14] Their role in infection is unclear, although it has been suggested that they may be involved in the initial stages of colonizing the upper respiratory tract.[5,12,15–17]

Studies indicate adhesin-encoding operons *pap*, *sfa,* and *afa* are prevalent in *E. coli* strains associated with urinary tract infections (pyelonephritis) in humans.[18,19] P fimbriae consist of a major fimbrial subunit, PapA, which determines 11 different serogroups, and a terminally located adhesin, PapG. Receptor specificity of P fimbriae is conferred by PapG, which recognizes different receptors of the globosides GbO_3 (globotriasylceramide), GbO_4 (globotetraosylceramide), and GbO_5 (globopentosylceramide) in P-blood group antigens of human and sheep erythrocytes.[20] The role of P adhesins in avian pathogenic *E. coli* has not been fully elucidated, but they appear to be involved in the colonization of internal organs.[8]

The afimbrial adhesin is a mannose-resistant, P-independent, X-binding adhesin, expressed by the *afa*-1 operon. It mediates specific binding to uroepithelial cell- and human erythrocyte-receptors. The nature of the receptor on the eucaryotic cell surface is not yet known.[21] The S fimbriae have a mannose-resistant adhesin, encoded by the *sfa* operon, that recognizes α-sialyl-β-2,3-galactose receptors, present on human and calf erythrocytes.[6,22] The presence of S fimbriae is also correlated with pathogenicity of *E. coli* in human meningitis and septicemia.[6,7,22] The role of afimbrial adhesin and S fimbriae in the pathogenicity of avian pathogenic *E. coli* remains unclear.

The purpose of this study was to compare the occurrence of *fim*, *pap*, *sfa*, and *afa* genes in *E. coli* strains isolated from cases of omphalitis, salpingitis, swollen head syndrome, and chronic respiratory disease in poultry.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

A total of 200 strains of *E. coli* were isolated from poultry in the state of São Paulo in Brazil. The strains were isolated from oviducts of broiler breeders with salpingitis (n = 50), yolk sacs of one-day-old chicks with omphalitis (n = 50), subcutaneous facial tissue of chickens with swollen head syndrome (n = 50), and air sacs from broilers with chronic respiratory disease (n = 50). Standard bacteriologic methods were used for isolation and identification of the organisms. All strains were stored at -20˚C in brain heart infusion broth (Difco) to which 15% glycerol was added after incubation. For adherence assays, bacterial strains were grown on colonization factor antigen agar and incubated at 37˚C for 18 to 24 hours.[23]

Tracheal Ring Cell Preparation and Adherence Assay

Tracheal sections were obtained from 10-day-old specific-pathogen-free chicks (Granja Rezende, Brazil) and cut into 4-mm sections.[17]Adherence tests were performed in 24-well, round-bottom microtiter plates. Three sections of trachea and Dulbecco's Modified Eagle Medium without calf serum were added to each well. The material was examined by inverse light microscopy for evidence of ciliary motility that would indicate cell viability. Bacterial strains plus tracheal rings were incubated at 37˚C for 30 minutes, after which they were washed six times with 50 mM (pH 7.4) phosphate-buffered saline and incubated for an additional 4 hours. The rings were fixed in 10% buffered formalin. A strain of *E. coli* K-12 was used as a negative control.[24]

Colony Hybridization

The test strains were examined by colony blot hybridization,[25] using specific DNA probes labeled with [α-^{32}P]-dATP by nick translation. The DNA probe used to detect *fim* B-H genes was a 9.6-Kb Hind III-Sal I fragment from recombinant plasmid pIB254.[26] Detection of *pap, afa,* and *sfa*

Table 1. Primers Obtained by Polymerase Chain Reaction for DNA Probes to Detect *pap, sfa* and *afa* Genes[39]

Gene	*E. coli* Strain	Oligonucleotide Primer Pairs (5´→3´)	Amplicon (bp)
pap	J96	GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GGA TGC AAT A	328
sfa	HB101 (pANN801–13)	CGG AGG AGT AAT TAC AAA CCT GGC A CTC CGG AGA ACT GGG TGC ATC TTA C	410
afa	KS–52	GCT GGG CAG CAA ACT GAT AAC TCT C CAT CAA GCT GTT TGT TCG TCC GCC G	750

Table 2. Analysis of *Esherichia coli* Isolates for Colony Hybridization and Tracheal Adherence

Source of *E. coli* Isolate*	Number of Isolates Positive for Given Gene by Colony Hybridization†			Number of Isolates Demonstrating Adherence to Tracheal Ring Epithelial Cells
	fim	*pap*	*sfa*	
Salpingitis	49	4[a]	2[a]	46
Omphalitis	48	4[a]	8[b]	50
Swollen head syndrome	47	14[b]	0[a]	50
Chronic respiratory disease	48	10[b]	2[a]	48
Total	**192**	**32**	**12**	**194**

*N = 50 per isolate.
†None of the isolates were *afa*$^+$ by colony hybridization.
[a,b]Data in columns with different superscripts are significantly different ($P < .05$)

genes involved oligonucleotide fragments obtained by polymerase chain reaction (Table 1). Positive and negative controls were included in all hybridization assays.

Statistical Methods

Differences between strains were tested by Fisher's exact test, using a Pearson statistic for data with low values (SPSS for Windows version 9.0; SPSS). Differences were considered to be significant when $P < .05$.

RESULTS

Results of tests for DNA hybridization and tracheal adherence are summarized in Table 2. The colony blot hybridization assay with the type-1 probe indicated the relevant sequence was present in 192 isolates (96%). There were no significant differences among the four disease groups in relation to the presence of the *fim* gene.

Of the 200 isolates evaluated, 32 (16%) carried the *pap* sequence and 12 (6%) carried the *sfa* sequence. Distributions of these genes in relation to the various diseases differed as follows: there were four *pap*$^+$ and eight *sfa*$^+$ isolates from the population of birds with omphalitis; four chickens with salpingitis were *pap*$^+$ and two were *sfa*$^+$; 10 *pap*$^+$ and two *sfa*$^+$ were detected among isolates from chickens with chronic respiratory disease; and 14 *pap*$^+$ were detected among isolates from swollen head syndrome. None of 50 isolates from swollen head syndrome samples was positive for *sfa*. The prevalence of the *pap* sequence in isolates from swollen head syndrome and chronic respiratory disease was significantly ($P < .05$) greater than that in isolates from salpingitis or omphalitis (Table 2). The prevalence of the *sfa* sequence was significantly ($P < .05$) greater in isolates from omphalitis than from any of the other isolates.

The concomitant presence of the gene sequences, *fim*$^+$ *pap*$^+$ and *fim*$^+$ *sfa*$^+$ is shown in Table 3. Only one isolate (0.5%) had *fim*$^+$, *sfa*$^+$, and *pap*$^+$ genes.

In the adherence assay, 194 strains (97%) were positive, including all isolates from omphalitis and swollen head syn-

Table 3. Distribution of *fim*, *pap* and *sfa*-related Nucleotide Sequences in Positive Isolates from Poultry Diseases

Genotype Pattern*	Number of Probe-Positive Isolates				
	Salpingitis	Omphalitis	Swollen Head Syndrome	Chronic Respiratory Disease	Total
fim$^+$	43	35	31	33	142
pap$^+$	0	0	2	1	3
sfa$^+$	0	1	0	0	1
fim $^+$ *pap*$^+$	3	4	12	9	28
fim$^+$ *sfa*$^+$	1	7	0	2	10
fim$^+$ *pap*$^+$ *sfa*$^+$	1	0	0	0	1
None	2	3	5	5	15
Total	**50**	**50**	**50**	**50**	**200**

* No isolates were positive for *pap*$^+$ *sfa*$^+$.

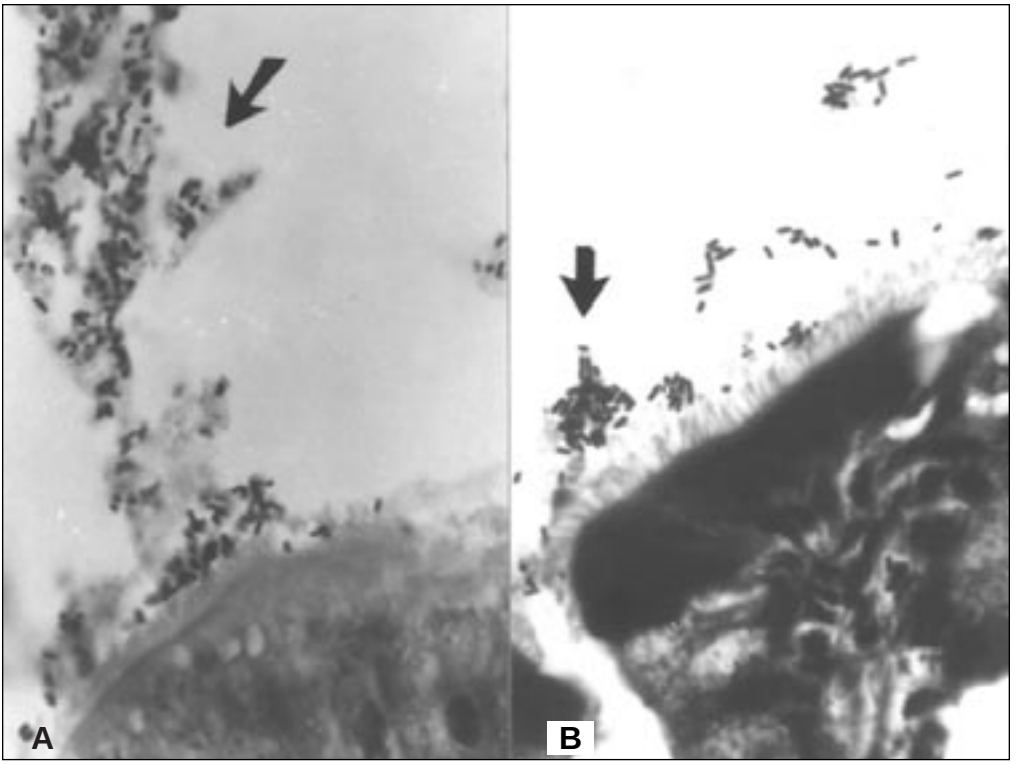

Figure 1. Histologic view of tracheal ciliated cells (**A**) showing bacterial adherence on epithelium and attachment in the mucous layer (*arrow*) for *E. coli* isolate EC 439 *fim*$^+$ from broiler breeders with salpingitis. Bacterial attachment with formation of microcolonies (*arrow*) for *E. coli* isolate EC 247 *fim*$^+$ from chicken with chronic respiratory disease (**B**).

drome, 92% for salpingitis, and 96% of the chronic respiratory strains (Figure 1). Histologic examination demonstrated the presence of a mucous layer as well as adherence of bacteria to ciliated cells (Figure 1).

DISCUSSION

Certain genotypic traits associated with *E. coli* extraintestinal disease in humans are frequently found in avian pathogenic *E. coli*. Epidemiologic investigations have shown a good correlation between the occurrence of

certain human diseases and the presence of specific virulence factors in *E. coli*.[27–29] Operons encoding P, S, and *afa* adhesins; production of α-haemolysin; and cytotoxic necrotizing factor type-1 contribute to the pathophysiology of urinary tract infections, whereas genes encoding for S fimbriae, K1 capsule, and Ibe 10 protein are correlated with the pathogenesis of neonatal meningitis.[30,31]

Avian pathogenic *E. coli* can be responsible for many localized and systemic diseases in poultry, but the pathophysiology of these infections remains unclear. In the present study, it was shown that the presence of *pap* and *sfa* genes varies among strains associated with different diseases. Findings in this study indicate that *pap* genes were detected with a higher frequency in swollen head syndrome and chronic respiratory disease isolates of *E. coli* (28 and 20%, respectively) than in omphalitis and salpingitis isolates (8%). Similarly, *sfa* genes were detected with a higher frequency in isolates from omphalitis (16%) than in strains from salpingitis and chronic respiratory disease (4%) and were absent from strains associated with swollen head syndrome. The data suggest that the role of these mannose-resistant fimbriae in mediating adherence of *E. coli* to different host tissues is a potential virulence factor in extraintestinal infections caused by avian pathogenic *E. coli*.

The study also confirms previous observations that mannose-sensitive adhesins are frequently present in avian pathogenic *E. coli*, and that type 1 fimbriae could play a role in tracheal colonization.[12,16,17,32–36] There was good correlation between adherence to tracheal sections and the presence of genes encoding for type 1 pili, although these fimbriae occur with similar frequency in strains from the four diseases studied. Pourbakhsh and coworkers[8] 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.

Using the tracheal ring-cell adherence model, it was possible to demonstrate adherence of the test organisms to epithelial cells and their presence in the mucus layer. Further studies are necessary to determine the role of mucus in the pathogenesis of respiratory and reproductive tract disorders, because the enhanced production of mucus could act as a primary factor in the development of disease. Catelli and coworkers[37] demonstrated that chickens experimentally infected with pneumovirus present with histopathologic lesions, including loss of epithelial cilia, hypertrophy of mucous glands, and increased mucus secretion, favoring a secondary infection by *E. coli* in swollen head syndrome. There is insufficient information in the literature to establish a correlation between the presence of type 1 fimbriae in avian pathogenic *E. coli* and penetration of mucus and ascending contamination of the vagina following cloacal colonization in breeder chickens. Nevertheless, such an 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 humans.[38] S fimbriae were rarely detected in avian pathogenic *E. coli*, and its role in the pathogenesis of avian colibacillosis is presently unclear. It is possible that bacteria with S fimbriae are of human origin, and chicks may become infected due to poor hygiene during handling of embrionated eggs in the hatchery.

CONCLUSIONS

Results of this study suggest that *pap* and *sfa* operon distribution can be varied among avian *E. coli* strains. The *pap* gene is observed more frequently in *E. coli* isolated from swollen head syndrome and chronic respiratory disease, whereas the *sfa* gene is observed with more frequency among omphalitis isolates. However, other studies should be carried out to establish the distribution of mannose-resistant adhesins in

pathogenic *E. coli*. Experimental infections will be useful for gaining an understanding of the role of fimbrial expression and the mechanisms of adhesion to specific tissues.

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