Receptor Binding Specificity and Pathogenicity of *Escherichia coli* F165-positive Strains Isolated from Piglets and Calves and Possessing *pap* Related Sequences

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ABSTRACT

Most of 82 F165-positive *Escherichia coli* isolated from calves and piglets with diarrhea or septicemia and possessing *pap* related sequences caused mannose-resistant, neuraminidase-resistant hemagglutination of human and bovine erythrocytes. Less than half of these isolates demonstrated binding specificity for the alpha-D-galactosyl-(1-4)-beta-D-galactopyranose or galactose-*N*-acetyl-alpha-(1-3) galactose-*N*-acetyl moieties recognized by P and F (or Prs) adhesins respectively. Binding specificity for the galactose-*N*-acetyl-alpha-(1-3) galactose-*N*-acetyl moiety was associated with isolates causing septicemia in newborn piglets.

RÉSUMÉ

La majorité des 82 souches de *Escherichia coli* F165-positives qui ont été isolées de veaux et porcelets souffrant de diarrhée ou de septicémie et qui possèdaient des séquences nucléotidiques reliées à l'opéron *pap* ont démontré un patron d'hémagglutination aux érythrocytes humains et bovins résistant au mannose et à la neuraminidase. Moins de la moitié de ces isolats ont démontré une spécificité de liaison au groupement alpha-D-galactosyl-(1-4)bêta-D-galac-topyrannose ou au groupement galactose-N-acétyl-alpha-(1-3)-galactose-N-acétyl qui sont des groupements reconnus par les adhésines P et F (ou Prs), respectivement. Une association entre la propriété de reconnaître le groupement galactose-N-acétyl-alpha-(1-3)-galactose-N-acétyl et la capacité de certains isolats à causer la septicémie chez les porcelets nouveau-nés a été démontrée.

Pathogenic bacteria may produce adhesins which are very often associated with fimbrial structures and are able to attach to erythrocytes. Fimbrial adhesins produced by extraintestinal *Escherichia coli* can be distinguished by their receptor specificity (1). P fimbriae which represent the main group of MR fimbriae associated with urinary tract infections, possess Pap G-adhesins which recognize the globoseries of glycolipids present on human erythrocytes with the P blood group antigen, the minimum binding receptor being the alpha-D-galactosyl-(1-4)-beta-D-galactopyranose (Gal-Gal) moiety, and are encoded by the *pap*, or *pap*-related, operons (2,3). The *prs* operon, which shows a high homology with the *pap* operon, encodes P fimbriae which possess the F (or Prs) adhesin (also called a Prs G-adhesin) that preferentially binds to the Forssman antigen, a major constituent on sheep, but not human, erythrocytes (3,4). The minimum binding receptor of the latter is the galactose-*N*-acetyl-alpha-(1-3) galactose-*N*-acetyl (Gal NAc-Gal NAc) moiety. Another important group of MR adhesins, the S fimbrial adhesins, are encoded by the *sfa* operon, recognize an alpha-sialyl-(2-3)-beta-Gal-containing receptor structure, and demonstrate neuraminidase-sensitive hemagglutination of bovine erythrocytes (5). S fimbriae are often produced by *E. coli* strains causing sepsis or newborn meningitis (6). In addition, nonfimbrial adhesive proteins of extraintestinal *E. coli*, such as the afimbrial adhesins (Afa), the latter being encoded by the *afa* operon, have been described (7). Nonhemagglutinating F1C fimbriae (pseudotype I) encoded by the *foc* operon, which shows a high homology with the *sfa* operon, have also been reported for *E. coli* strains in urinary tract infections (8).

The F165 fimbrial complex has been found on *E. coli* isolates from piglets and calves with septicemia and/or diarrhea (9,10). The purified F165 fimbrial complex possesses at least two separate major protein subunits of 19 kDa and 17.5 kDa (11) which we have subsequently designated $F165_1$, and $F165_2$ respectively. The $F165_1$ fimbrial component is encoded by a *prs*-like operon, recognizes the Gal NAc-Gal NAc moiety, and hemagglutinates sheep erythrocytes (12). The $F165_2$ fimbrial component is similar, but not identical, to the F1C fimbrial component (13). We have demonstrated that *pap*-, *afa*- and *sfa*- related nucleotide sequences are present in many of the porcine and bovine F165-positive isolates (14). We now report on the receptor binding specificity of the adhesins produced by these strains and on the relationship between presence of these adhesins and pathogenesis of septicemia in pigs.

Eighty-two *pap*+ *E. coli* isolates producing the fimbrial antigen complex F165 were selected for this study (14). Certain of these strains were also either *afa*+ or *sfa*+. These strains had been isolated between 1984 and 1987 at the Faculté de médecine vétérinaire, Saint-Hyacinthe from calves or piglets with septicemia and/or diarrhea. Mannose sensitive

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TABLE I. Relationships among hemagglutinins, P and F adhesin expression and presence of *pap*, *afa*, or *sfa* genes in F165-positive *E. coli*

Fimbrial genotype	No. of isolates positive: Total	MRHA of erythrocytes from: Human OP_1	Bovine	Sheep	Agglutination: P-latex[a]	F-latex[b]	P- and F-latex
pap	49	38	44	4	12	7	11
pap + *afa*	24	22	22	0	6	5	6
pap + *sfa*	9	0	0	6	0	5	1

[a]Latex beads coated with Gal-Gal
[b]Latex beads coated with Gal NAc-Gal NAc

TABLE II. Adhesin genotype and receptor binding specificity of F165-positive *pap* + *E. coli* isolates causing septicemia in colostrum-deprived newborn piglets

Pathogenicity in pigs	No. of isolates Examined	Genotype *afa*+	*sfa*+	Agglutination positive: P-latex[a]	F-latex[b]	Bovine erythrocytes[c]
Septicemia	13	6	2	5	10[d]	10
No septicemia	5	1	0	2	0	5

[a]Latex beads coated with Gal-Gal
[b]Latex beads coated with Gal NAc-Gal NAc
[c]MRHA of bovine erythrocytes
[d]Two isolates showed MRHA of sheep erythrocytes

hemagglutination (MSHA) and mannose resistance hemagglutination (MRHA) were performed essentially as described (10). Tests to demonstrate MRHA were performed after growth of bacteria on minimal Davis agar (Difco Laboratories, Detroit, Michigan) plus casamino acids (MD-1 agar) at 37°C (10,11). Hemagglutination of bovine erythrocytes was repeated after treatment of erythrocytes with neuraminidase at a concentration of 50 mU/mL for 20 min at 37°C as described (15). Latex beads (20 μL) coated with Gal-Gal (P1 disaccharide-Latex, Chembiomed Ltd., Edmonton, Alberta) or Gal NAc-Gal NAc (Forssman disaccharide Latex, Chembiomed Ltd), were mixed on a slide with an equal volume of bacterial suspensions at 4°C. After rocking for 2 min, agglutination was read.

Strains were tested for pathogenicity by intragastric inoculation of hysterectomy-derived, colostrum-deprived newborn pigs (16). All experimental procedures were carried out following the guidelines of the Guide to the Care and Use of Experimental Animals.

Less than half of the isolates demonstrated specificity for the Gal-Gal (36/82) or Gal NAc-Gal NAc (35/82) moieties that are usually recognized by P or F adhesins (Table I). Most *pap*+, *afa*+ isolates, regardless of Gal-Gal or Gal NAc-Gal NAc binding specificity, agglutinated human OP_1 (22/24) and bovine (22/24) erythrocytes. Two major patterns of MRHA were observed for *pap*+, *afa*−, *sfa*− isolates: those agglutinating human OP_1 and bovine erythrocytes and those agglutinating only bovine erythrocytes. The MRHA of bovine erythrocytes was not inhibited in the presence of neuraminidase. In contrast, none of the nine *pap*+, *sfa*+ isolates agglutinated human OP_1 or bovine erythrocytes. However, these isolates demonstrated Gal NAc-Gal NAc binding specificity, and they also agglutinated sheep erythrocytes. Very few *pap*+ or *pap*+, *afa*+ isolates demonstrating binding specificity for the Gal NAc-Gal NAc moiety (2/29) also agglutinated sheep erythrocytes, suggesting that at least two classes of adhesin recognizing this moiety are present in F165-positive isolates.

Eighteen serum-resistant, aerobactin-positive isolates were examined for pathogenicity in newborn pigs (two animals/isolate). Thirteen isolates caused septicemia in 2/2 inoculated pigs. Affected pigs became depressed and weak and either died or were euthanatized in a moribund state, 36 to 48 h after inoculation. At necropsy, all pigs inoculated with septicemic isolates had lesions of fibrinous polyserositis. Five isolates caused no signs of septicemia for at least four days after inoculation of pigs. All isolates demonstrating Gal NAc-Gal NAc receptor specificity, of which two isolates showed MRHA of sheep erythrocytes, induced septicemia in inoculated pigs (Table II). Isolates demonstrating Gal-Gal receptor specificity only induced septicemia in inoculated pigs when Gal NAc-Gal NAc specificity was also present. Similarly, isolates demonstrating MRHA of bovine erythrocytes only induced septicemia when Gal NAc-Gal NAc specificity was also present. Both *sfa*+ isolates and six of seven *afa*+ isolates were septicemic in pigs.

Our results demonstrate that F165-positive *pap*+ isolates may express one or more of at least three adhesins of different receptor specificities based on agglutination of erythrocytes from different animal species and of latex beads coated with either the Gal-Gal or Gal NAc-Gal NAc. Certain isolates possessing both the *pap* and the *sfa* genes manifested a completely different receptor specificity to those possessing the *pap* gene alone or the *pap* and *afa* genes. These isolates agglutinated sheep but not OP_1 human erythrocytes and latex beads coated with Gal NAc-Gal NAc, a phenotype which closely resembles that of the F adhesin (4,5). Certain *pap*+ or *pap*+, *afa*+ isolates also agglutinated latex beads coated with Gal NAc-Gal NAc, but did not agglutinate sheep erythrocytes. This phenotype could represent the expression of an adhesin similar to that of F-fimbriae, but with certain chemical and structural differences which do not allow it to recognize the receptor on sheep erythrocytes. Certain *pap*+ or *pap*+, *afa*+ isolates agglutinated latex beads coated with Gal-Gal, demonstrated the bovine erythrocyte MRHA pattern, and sometimes agglutinated latex beads coated with Gal NAc-Gal NAc. These isolates probably produce fimbriae, coded by the *pap* gene, with a similar receptor specificity to those P fimbriae produced by uropathogenic *E. coli* isolates from individuals with pyelonephritis. Finally, certain isolates hemagglutinated bovine erythrocytes and did not demonstrate Gal-Gal

or Gal NAc-Gal NAc receptor specificity. The adhesin responsible for MRHA of bovine erythrocytes by the F165-positive isolates differs from P fimbriae which do not agglutinate erythrocytes of this species (3) and S fimbriae which cause neuraminidase-sensitive agglutination of bovine erythrocytes (5). A multiplicity of adhesins could be necessary for recognition of various receptors during the course of extraintestinal disease and may help to increase the pathogenicity of a given *E. coli* strain. Alternatively, these adhesins may have no role in pathogenicity but are coexpressed with other virulence determinants. In our pig infection studies, we found that isolates causing septicemia generally expressed two or more adhesins and often possessed *afa*-related DNA sequences. In contrast, nonsepticemic isolates mostly were *afa*- and *sfa*-negative and did not recognize Gal-Gal. However, no relationship was observed between pathogenicity and ability of isolates to hemagglutinate bovine erythrocytes.

All isolates recognizing Gal NAc-Gal NAc induced septicemia in newborn pigs. We have now demonstrated that a nonhemagglutinating, fimbriae-negative, Gal NAc-Gal NAc-negative Tn*phoA* mutant of such an isolate is no longer pathogenic in newborn pigs (unpublished results). It is possible that expression of an adhesin with this receptor specificity confers on bacteria the ability to withstand nonspecific host defence mechanisms and eventually reach sufficient numbers to cause septicemia in the pig.

In conclusion, we have demonstrated that *pap*+ F165-positive isolates express one or more adhesins with receptor binding specificities for Gal-Gal (P adhesin), Gal NAc-Gal NAc (F adhesin), and a surface antigen found on bovine and human erythrocytes. Our data demonstrate the multiplicity of adhesins associated with septicemic F165-positive isolates and suggest that the presence of at least one, the F adhesin, may contribute to the development of septicemia in newborn pigs. We are currently investigating this possibility.

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