

## Analysis of the F Antigen-Specific *papA* Alleles of Extraintestinal Pathogenic *Escherichia coli* Using a Novel Multiplex PCR-Based Assay

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**Polymorphisms in PapA, the major structural subunit and antigenic determinant of P fimbriae of extraintestinal pathogenic *Escherichia coli*, are of considerable epidemiological, phylogenetic, and immunotherapeutic importance. However, to date, no method other than DNA sequencing has been generally available for their detection. In the present study, we developed and rigorously validated a novel PCR-based assay for the 11 recognized variants of *papA* and then used the new assay to assess the prevalence, phylogenetic distribution, and bacteriological associations of the *papA* alleles among 75 *E. coli* isolates from patients with urosepsis. In comparison with conventional F serotyping, the assay was extremely sensitive and specific, evidence that *papA* sequences are highly conserved within each of the traditionally recognized F serotypes despite the diversity observed among F types. In certain strains, the assay detected serologically occult copies of *papA*, of which some were shown to represent false-negative serological results and others were shown to represent the presence of nonfunctional *pap* fragments. Among the urosepsis isolates, the assay revealed considerable segregation of *papA* alleles according to O:K:H serotype, consistent with vertical transmission within clones, but with exceptions which strongly suggested horizontal transfer of *papA* alleles between lineages. Sequencing of *papA* from two strains that were *papA* positive by probe and PCR but F negative in the new PCR assay led to the discovery of two novel *papA* variants, one of which was actually more prevalent among the urosepsis isolates than were several of the known *papA* alleles. These findings provide novel insights into the *papA* alleles of extraintestinal pathogenic *E. coli* and indicate that the F PCR assay represents a versatile new molecular tool for epidemiological and phylogenetic investigations which should make rapid, specific detection of *papA* alleles available to any laboratory with PCR capability.**

P fimbriae, the principal mannose-resistant adherence organelles of extraintestinal pathogenic *Escherichia coli*, mediate Gal( $\alpha$ 1-4)Gal-specific binding to glycolipid isoreceptors on host epithelial cells, thereby contributing to pathogenesis by promoting bacterial colonization of host tissues and stimulating an injurious host inflammatory response (11, 33, 34). P fimbriae are antigenically diverse, occurring in 11 known serological variants, which are termed F7-1, F7-2, and F8 to F16 according to the system of Ørskov et al. (18, 64, 68, 70). Whereas Gal( $\alpha$ 1-4)Gal-specific binding is mediated by PapG, the fimbrial tip adhesin molecule (29, 56), the antigenic diversity of P fimbriae is attributable to peptide sequence variability within PapA, the major P fimbrial structural subunit (12, 13, 22, 55, 57, 96). PapA is present in hundreds to thousands of identical copies per fimbria and is encoded by the corresponding gene, *papA* (12, 57). Wild-type *E. coli* strains can contain up to three copies of the *pap* operon and, since each *pap* operon can have a different variant (allele) of *papA*, can express up to three different P fimbrial (F)-antigen types each (1, 18, 67, 68, 70, 75–77).

The evolution of antigenic diversity within PapA may have been driven by selective pressure from the host immune system (8, 12, 96). P fimbriae are expressed in vivo within the urinary tract during infection and appear to be physiologically relevant immunogens (2, 17, 49, 54, 73, 87). They also are effective antigenic targets for a protective host humoral immune response (48, 52, 71, 81). Thus, avoidance of the host immune attack would be predicted to favor a diverse PapA antigenic repertoire in the *E. coli* population as a whole and even within individual strains, which may be able to switch the expression of their various *papA* alleles on or off through phase variation (62, 68, 78, 94).

Knowledge of the F antigen status of wild-type *E. coli* strains can be valuable in several ways. First, independent of its pathogenetic significance, the F antigen can serve as a typing tool to help differentiate strains, much as do the O, K, and H antigens of conventional *E. coli* serotyping (18, 65, 70). Second, since F antigens are markers for the corresponding *papA* alleles, they can be used to trace the vertical or horizontal transmission of particular *pap* variants within the *E. coli* population, e.g., as defined by O:K:H serotypes, and in relation to other virulence factors (61, 70, 72, 74). Third, epidemiological analyses of F types among strains from defined clinical syndromes or host populations can reveal the clinical associations of particular F

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variants, which could help guide future efforts to develop and deploy an anti-PapA vaccine (12, 63, 64).

The current "gold standard" method for F determination involves rocket immunoelectrophoresis and crossed-line immunoelectrophoresis (67, 68), demanding techniques which require highly specialized equipment, skills, and F-specific antisera; hence, at present it is largely confined to a single laboratory (88). Consequently, few studies have been done regarding associations of F antigens with other bacterial properties, clonal markers, or clinical syndromes. F-specific monoclonal antibodies, which can be used in (more widely available) enzyme-linked immunosorbent assays (68), have been developed, but they have been developed for only a subset of the known F types (1, 15–18, 68) and have not come into general use.

Previous efforts to exploit DNA sequence polymorphisms for typing of *papA* variants by restriction endonuclease analysis (19) or with a battery of oligonucleotide probes (13) were handicapped by the unavailability of sequence data for the full range of *papA* alleles. Full-length sequencing of *papA* from wild-type strains, although highly informative and requiring no prior knowledge of existing *papA* sequences (8), is impractical for large-scale screening. The recently completed cloning and sequencing of *papA* variants corresponding to all 11 recognized F types of P fimbriae (7) suggested the possibility of exploiting amplification technology for *papA* allele determinations. In the present study, we sought to develop and validate a PCR-based assay for the 11 known F-type-specific *papA* alleles of *E. coli* and to use this assay to investigate the *papA* allele repertoire of a collection of well-characterized blood isolates from patients with urosepsis.

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## MATERIALS AND METHODS

**Bacterial strains.** Control strains used in derivation and validation of the PCR assay included diverse wild-type strains plus, in many instances, their recombinant *pap* derivatives (e.g., Tables 1 and 2). The derivation set used in assay development consisted mainly of the source strains for the F type-specific *papA* sequences on which the PCR assay was based. The derivation set strains (and their respective F types and wild-type parent strains) were HB101/pPIL110-75 (F7-1, from AD110) (99), HB101/pPIL110-75 (F7-2, from AD110) (95), AM1727/pANN921 (F8, from 2980) (25), AM1727/pPIL288-10 (F9, from C1018) (14), MOSBLUE/pF10 (F10, from C1960-79) (this study), AM1727/pPIL291-15 (F11, from C1976) (15), 1442 (F12, wild type) (22), HB101/pRHU845 (F13, from J96) (30), MOSBLUE/pF14 (F14, from C1023-79) (this study), MOSBLUE/pF15 (F15, from C1805-79; present study), and MOSBLUE/pF16 (F16, from C83-83) (this study). The first validation set (Table 1) consisted primarily of strains from the International *Escherichia* and *Klebsiella* Center (IEKC), World Health Organization, Copenhagen, Denmark, and included, for most F types, that laboratory's type strain for the particular F type. These were supplemented with diverse other F-typed strains, as available. The second validation set (Table 2) consisted of nine strains, generously provided by Han de Ree, for which the O:K and F serotypes had been previously published (16–18, 68).

The application set consisted of 75 well-characterized blood culture isolates of *E. coli* collected from adults with urosepsis in Seattle, Wash., in the mid-1980s. The status of these strains with respect to multiple characteristics, including *pap*, diverse other virulence factors, O:K:H serotype, carboxylesterase B electrophoretic type, and host compromise status, has been reported previously (35, 39–42). Strains were considered to belong to a particular O:K:H serotype if they exhibited any two of the three corresponding antigens plus no other O, K, or H antigen (41).

**Sequencing of *papA* alleles.** The nucleotide sequences of the F7-1, F7-2, F9, F11, F12, and F13 *papA* variants were as previously published (4, 22, 93, 97, 98) and as found in the GenBank database under accession no. X02921 (F7-1), M12861 (F7-2), M68059 (F9), L07420 (F11), X62157 (F12), and X61239 (F13). The nucleotide sequences of the F8, F10, F14, F15, and F16 *papA* variants were experimentally determined. *papA* was amplified from control strains AM1727/pANN921 (F8 clone, derivation set), C1960-79 (F10 wild type, Table 1), C1023-79 (F14 wild type, Table 1), C1805-79 (F15 wild type, Table 1), and C83-83 (F16 wild type, Table 1) using primers 5'-ctgagaattcaggtgaaattcgc-3' (forward) and 5'-atgatgaattcgtttatgccggtgccg-3' (reverse), which are modified from the corresponding *pap* sequences to provide *EcoRI* sites for cloning. The resulting *papA* amplicons were electrophoresed in agarose gel, and the DNA

fragments were removed from the gel using the GeneClean Kit (Bio 101, Inc., La Jolla, Calif.). Purified fragments were ligated into the TA cloning vector (Invitrogen, Groningen, The Netherlands) to create plasmids pF8, pF10, pF14, pF15, and pF16, respectively, and transformed into the electrocompetent cells of the TA cloning kit to create the corresponding recombinant *papA* control strains. Sequencing of the inserts was done with the dideoxynucleotide chain termination method (88) using the Automated Laser Fluorescence sequencer (Pharmacia, Uppsala, Sweden). The M13 universal and reverse sequencing primers labeled with Cy5 were used in the AutoRead 1000 Sequencing Kit (Pharmacia, LKB Biotechnology). Three different clones of each fragment were sequenced.

**Sequence alignments and dendrogram construction.** To illustrate the phylogenetic relationships between the PapA variants, an alignment of the predicted mature peptides was made using the CLUSTAL-X program (26). The alignment was then used to calculate a distance matrix from which an unrooted phylogenetic tree was inferred according to the neighbor-joining method (86) using the PHYLIP 3.5c package (21) (Fig. 1). (J. G. Kusters, Department of Medical Microbiology, Free University, Amsterdam, The Netherlands, generously constructed the tree.) For comparison with PapA, the two most closely related known pilin genes from other members of the family *Enterobacteriaceae*, i.e., MR/P of *Proteus mirabilis* (5, 6) and SMF of *Serratia marcescens* (58), were included in the tree (7) (Fig. 1).

**F PCR primers.** A universal forward F primer (Ff [5'-ggcagtggtcttttgg-3']) was selected from the consensus signal sequence region of *papA*, without regard for peptide structure. To guide selection of immunologically relevant and F-type-specific reverse primers, secondary-structure predictions were made for the 11 translated F-specific PapA peptides using the PlotStructure and PeptideStructure components of the Wisconsin Package (version 5.0; Genetics Computer Group, Madison, Wis.) (32). For each of the 11 PapA sequences, unique peptide regions were identified that were predicted to be hydrophilic (27, 28), surface exposed (20), and antigenic (32) and to contain beta (reverse) turns (9, 23), hence, to be putative F-specific epitopes (89). Candidate reverse primers for each F type were selected from the coding regions corresponding to these putative F-specific epitopes. Since all putative F-specific epitopes for the F12 peptide had corresponding DNA sequences which were suboptimal for use as primers, two compromise F12 reverse primers were chosen. One primer (F12/F15r) corresponded to a predicted antigenic epitope shared with F15, whereas the other (F12r) was unique to F12 at the nucleotide level but corresponded to a predicted nonantigenic peptide region shared by the F12 and F16 variants (not shown).

Reverse primers were sorted into three pools of four primers each while maintaining within each pool compatibility between primers and a distribution of product sizes that would allow ready resolution of products in agarose gels (Fig. 2 and 3). Reverse primers were tested in combination with the universal forward primer (Ff), first individually and then in multiples, against single and multiple positive and negative control DNAs. PCR conditions and primer sequences were adjusted as needed to achieve balanced and specific amplification of all F variants with the derivation set strains (Fig. 3).

**F PCR methods.** Amplification was done in a 25- $\mu$ l reaction mixture containing template DNA (2  $\mu$ l of boiled lysate [37]), 4 mM MgCl<sub>2</sub>, the four deoxynucleoside triphosphates at 0.8 mM each, 0.6  $\mu$ M concentration of each primer (except for those marked with an asterisk below, which were used at a concentration of 0.3  $\mu$ M), and 2.5 U of AmpliTaq Gold in 1 $\times$  PCR buffer (Perkin-Elmer, Branchburg, N.J.). The three primer pools, with the reverse primers listed in order of decreasing amplicon size within each pool, were as follows: pool A, \*F15r (gtcacatttggccactgc; 455 bp), F13r (gggtattgatcacctctcg gag; 400 bp), F14r (gcagcatatctttattgtccc; 320 bp), and \*F11r (ggcccagtaaaagat aattgaacc; 277 bp); pool B, \*F9r (aagcggcccttgacgcttt; 416 bp), \*F7-1r (ttcacc gttttccactcg; 375 bp), \*F8 (gtaccactacagactctgg; 251 bp), and \*F12/15r (aattct ggccgtgaggatgaca; 179 bp); pool C, \*F12r (cccatcagacagactgaca; 393 bp), F10r (ctctcattatgaccagaacct; 312 bp), F16r (gttccgctttattaccagc; 239 bp), and F7-2r (tttgggtgacttcccacc; 183 bp). All three primer pools also contained the forward primer Ff (Fig. 2).

The reaction mixture was heated to 95°C in an automated thermal cycler (PTC-100-96; MJ Research, Watertown, Mass.) for 12 min to activate the AmpliTaq Gold. This initial denaturation was followed by 25 cycles of denaturation (94°C, 30 s), annealing (67°C, 1 min), and extension (72°C, 1.5 min) and a final extension step (72°C, 10 min). Samples were electrophoresed in 2% agarose gels, stained with ethidium bromide, destained with distilled water, and then photographed using a UV transilluminator and digital capture system (Gel Doc; Bio-Rad, Hercules, Calif.). The sizes of the amplicons were determined by comparing them to a 100-bp DNA ladder (Gibco-Bethesda Research Laboratories, Gaithersburg, Md.) which was run on the same 2% agarose gel (Fig. 2 and 3).

**Confirmation of specificity of F PCR products.** To confirm that a PCR product of a size corresponding to one of the predicted F-specific amplicons actually represented that *papA* variant, the nucleotide sequence of at least one putative F-specific amplicon was determined for each F type as derived from a strain other than the source strain for the corresponding type-specific *papA* sequence (Table 3). Amplicons were purified using the QIAquick PCR purification kit (Qiagen Inc., Chatsworth, Calif.) or MicroSpin S-3000 HR columns (Amersham Pharmacia Biotech, Piscataway, N.J.) and subsequently directly sequenced by the dideoxy-chain termination method (88). Experimentally determined sequences were compared with known *papA* sequences by using the BLAST algorithm (3)

TABLE 1. First validation set strains for F PCR assay

F serotype(s)		F PCR result	Strain name	O:K:H serotype (or source strain, if clone)	Source or reference
Putative <sup>a</sup>	Confirmatory				
F7-1, F7-2	ND <sup>b</sup>	F7-1, F7-2	AD110	O6:K2:H1	18
F7-1 <sup>d</sup>	ND	F7-1	MC400/pDAL201B	(AD110)	55
F7-2 <sup>d</sup>	ND	F7-2	HB101/pDAL210B	(AD110)	55
F7-1, F7-2	ND	F7-1, F7-2	CFT073	O6:K2:H1	60
F7-1	ND	F7-1	DH5 $\alpha$ /pKgg201	(CFT073)	60
F7-2	ND	F7-2	DH5 $\alpha$ /pDIW101	(CFT073)	60
F7-2	ND	F7-2	C952-79	O6:K2:H1	IEKC
F7-2	ND	F7-2	C953-79	O6:K2:H1	IEKC
F7-2	ND	F7-2	C1898-79	O6:K2:H1	IEKC
F7-2	ND	F7-2	2H4	O6:K2:H1	41
F8	F8, F10	F8, F10	C1253-77	O18ac:K-:H-	IEKC
F8	F8	F8, F10	C1254-77	O75:K-:H5	IEKC
F8	F8, F10	F8, F10	C351-82	O18ac:K-:H-	IEKC
F8	F8, F10	F8, F10	C405-82	O18ac:K-:H5	IEKC
F8	F8, F10 <sup>e</sup>	F8, F10	C805-83	O18ac:K-:H-	IEKC
F8	F <sup>-</sup>	F10	C659-81	O1:K51:H-	IEKC
F8	F8	F8, F10	C321-82	O18ac:K-:H-	IEKC
F8	F8	F8, F10	C825-83	O75:K5:H-	IEKC
F8	F8, F12	F8, F12	V30b	O16:K1:H-	41
F9	F9	F9, F10	3669	O2:K5:H4	12
F9 <sup>d</sup>	ND	F9	HB101/pDAL200A	(3669)	12
F9	F9	F9, F10	C1018-79	O2:K5:H4	IEKC
F9	F9	F9, F10	C481-82	O1:K1:H-	IEKC
F9	F9, F10 <sup>e</sup>	F8, F9	C483-82	O1?:K1:H-	IEKC
F9	F14, F16	F13, F14, F16	C878-83	O4:K12:H5	IEKC
F10	ND	F10	C1960-79	O7:K1:[H1]	IEKC
F10	ND	F10	C997-79	O7:K1:H-	IEKC
F10	ND	F10	C328-82	O7:K1:H-	IEKC
F10	ND	F10	C906-83	O7:K1:H-	IEKC
NA <sup>c</sup>	F <sup>-</sup>	F10	G1062a	O4:K7:HR	38
F11	F11, F16 <sup>e</sup>	F11, F16	IA2	O4:K12:H-	10
F11	ND	F11	HB101/pDC1	(IA2)	16
F11	ND	F11	C1976-79	O1:K1:H7	IEKC
F11	ND	F11	C974-79	O1:K1:H7	IEKC
F11	F10 <sup>e</sup> , F11	F10, F11	V31	O4:K12:H-	41
F11, F16	ND	F11, F16	AFR015	O4:K+:H-	45
F11, F16	F10 <sup>e</sup> , F11, F16	F10, F11, F16	BOS040	O4:K+:H1	45
NA	F10, F11, F16	F10, F11, F16	JR1	O4:K7:H1	82
F12-1, F12-2	ND	F12	C1979-79	O16K1:H-	IEKC
F12-2	ND	F12	C493-82	O6:K13:H1	IEKC
F12-2	ND	F12	C438-82	O75:K5:H5	IEKC
F12-2	ND	F12	C469-82	O75:K95:H5	IEKC
F13	ND	F13	J96	O4:K-:H5	44
F13	ND	F13	DH5 $\alpha$ /pJJ48	(J96: allele I)	47
F13	ND	F13	HB101/pJFK102	(J96: allele III)	51
F13	F13, F14	F13, F14	CP9	O4:K10,K54/96:H5	44
NA	ND	F13	NM554/pCP9I	(CP9)	This study
NA	ND	F14	NM554/pCP9III	(CP9)	This study
F13	F13, F14	F13, F14	BF1023	O4:K10,K54/96:H5	44
F13	F13, F14	F13, F14	BF1056	O4:K10,K54/96:H5	44
F13	F13, F14	F13, F14	BOS038	O4:K10,K54/96:H5	44
F13	F13, F14	F13, F14	BOS110	O4:K10,K54/96:H5	44
F13	F13, F14	F13, F14	518	O4:K10,K54/96:H5	45
F13	ND	F13, F14	18878	O4:K10,K54/96:H5	IEKC
F13	ND	F13	BF1040	O4:K3:H5	44
F13	ND	F13	BF9043	O4:K3:H5	44
F13	ND	F13	CA002	O4:K3:H5	45
F13	ND	F13	CA062	O4:K3:H5	45
F13	ND	F9, F13	CA022	O4:K3:H5	45
F13	F11, <sup>e</sup> F13	F11, F13	R28	O4:K3:H5	45
F13	F13, F16	F13, F16	3048	O4:K-:H-	31

Continued on following page

TABLE 1—Continued

F serotype(s)		F PCR result	Strain name	O:K:H serotype (or source strain, if clone)	Source or reference
Putative <sup>a</sup>	Confirmatory				
F13, 16	ND	F13, 14, 16	C134-73	O4:K12:H5	IEKC
F13, F14, F16	ND	F13, F14, F16	20025	O4:K12:H-	IEKC
F14	ND	F14	C127-86 <sup>f</sup>	(Unknown)	IEKC
F14	ND	F14	C 818-83	O25:H9	IEKC
F14	ND	F14	C1023-79	O83:K24:H31	IEKC
F15	ND	F10, F15	C1805-79	O75:K5:H-	IEKC
F15	F <sup>-</sup>	F10	C826-83	O75:K5:H-	IEKC
F15	F10 <sup>e</sup> , F15	F10, F15	C372-82	O75:K5:H+	IEKC
F15	F10, F15	F10, F15	C394-82	O75:K5:H-	IEKC
F15	F10 <sup>e</sup> , F15	F10, F15	C312-82	O75:K5:H-	IEKC
F16	ND	F16	C83-83	O157:K-:H45	IEKC
F16	F14, F16	F14, F16	PM8	O4:K12:H-	41
F16	F16	F7-1, F16	BOS021	O4:K+:H-	45
F16	ND	F16	BOS046	O4:K12:H-	45

<sup>a</sup> According to records of the IEKC.

<sup>b</sup> ND, not done.

<sup>c</sup> NA, not applicable.

<sup>d</sup> F type according to reference 12.

<sup>e</sup> Antigen detected only in second or third round of confirmatory serotyping.

<sup>f</sup> Recombinant.

(<http://www.ncbi.nlm.nih.gov/BLAST/>) to find the closest match in the GenBank, EMBL, DDBJ, and PDB sequence data banks.

**Detection of *papG* alleles, other *pap* elements and virulence factor genes, hemolysin, and hemagglutination.** In selected strains, the three *papG* alleles were detected using an established allele-specific PCR assay (39). Other *pap* elements and non-*pap* virulence factors were detected by PCR and/or by dot blot hybridization (under high-stringency conditions) by using primers, probes, and experimental conditions previously described (43, 46). Hemolysin production was assessed by growth on 5% sheep blood agar. Mannose-resistant hemagglutination (MRHA) was assessed using human A<sub>1</sub>P<sub>1</sub> and sheep erythrocytes, with pigeon egg white used as a specific inhibitor of P fimbriae to differentiate P pattern MRHA from non-P MRHA, as previously described (36).

**Cosmid cloning of *papG* operons from strain CP9.** To isolate each of the two *pap* operons from control strain CP9 (Table 1) for separate analysis, transformants of *pap*-negative K-12 host strain NM554 containing a previously constructed cosmid library from strain CP9 (85) were incubated with human or sheep erythrocytes at 4°C in excess α-methyl-D-mannose to allow selective hemadsorption of bacterial clones expressing PapG allele I or III, respectively (38). Following hemadsorption, erythrocytes were extensively washed and then plated on selective agar. Colonies from overnight growth at 37°C which exhibited the appropriate MRHA phenotype were confirmed as containing the appropriate *papG* allele by PCR (37).

**Sequencing of novel *papA* variants.** Certain strains were positive for *papA* by dot blot assay and by flanking-primer PCR but were negative with the F PCR

assay. Consequently, *papAH* amplicons from these strains (generated using forward consensus primer Ff and reverse primer PapAr [5'-cgcccaccatcgtctcttc-3'] which is from the 5' end of *papH*) were directly sequenced as described above for putative F-specific amplicons. Sequences were analyzed as described above for other *papA* sequences. A specific reverse primer (F48r [5'-gttcattggctggatt g-3']) which was compatible with the pool A reverse primers was designed for one of these new *papA* variants (F48) using the approach described above for the other reverse primers and was used to screen selected strains in combination with the universal forward primer Ff.

**RAPD fingerprinting.** Random amplified polymorphic DNA (RAPD) genomic fingerprints were generated for selected strains using boiled bacterial lysates as template DNA, Ready-To-Go PCR beads (Pharmacia), decamer primer 1290, and amplification conditions previously described (100). Fingerprints were compared side by side in ethidium bromide-stained 1% agarose gels.

**Serological methods.** Historical O:K:H and F types were from the literature or from the records of the IEKC. Confirmatory serotyping was done for selected strains as part of the present study by the IEKC. Lipopolysaccharide (O), capsular (K), and flagellar (H) antigens were determined by using the established typing sera and methods specified by Ørskov and Ørskov (66). F determination was done by using rocket immunoelectrophoresis, followed by crossed-line immunoelectrophoresis, as previously described (68). For selected strains, 10× concentrated F10 antiserum was used to enhance detection of the F10 antigen.

**Statistical methods.** Comparisons of proportions were tested using Fisher's exact test with a significance threshold of  $P < 0.05$ .

TABLE 2. Second validation set of strains for F PCR assay

Strain <sup>a</sup>	Published O:K serotype	Confirmatory O:K:H serotype	Published F type	Confirmatory F serotyping result			F PCR result
				Round 1	Round 2	Round 3	
AD303	O25:K-	O4:K2:H1	F11, F16	F11	F10, F11	F10, F11, F16	F10, F11, F16
AD309	O77:K-	O77:K16:H4	F7-1, F11, F12	F11, F12	ND <sup>d</sup>	ND	F11 <sup>b</sup>
AD314	O4:K12	O4:K12:H1	F11, F16	F11	F10?, F11	F11, F16	F11, F16
NS3	O14:K+	OR:K?:H6	F7-1, F13, F14	F7-2, F13	ND	ND	F7-2, F13
NS24	O1:K1	O1:K1:H7	F7-1, F11	F11	ND	ND	F11
NS25	O4:K12	O4:K12:H1	F11, F16	F11	F10, F11, F16?	F10, F11, F16	F10, F11, F16
NS26	O83:K24	O83:K-:H31	F7-2, F14	F14	ND	ND	F14
SP57	O157:K-	O157:K-:H45	F16 + ?	F <sup>-</sup>	F10?, F16	F16 <sup>c</sup>	F16
SP88	O157:K-	O157:K-:H45	F16 + ?	F <sup>-</sup>	F10?, F16	F16	F16

<sup>a</sup> Sources of strains and serotypes: AD309, AD314, NS3, NS24, NS25, and NS26, reference 18; SP57 and SP88, reference 68.

<sup>b</sup> Subsequently found to be PCR positive for the new F48 *papA* allele (but still PCR negative for F12).

<sup>c</sup> A second colony type noted in the serotyping laboratory was serotyped as OR:K-:H45, F10, F16. (This variant was not retested with the F PCR assay.)

<sup>d</sup> ND, not done.

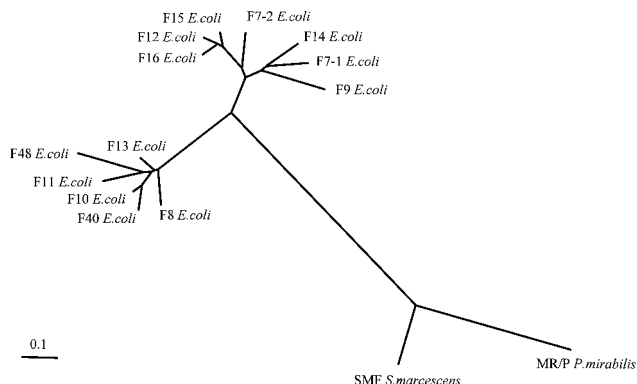


FIG. 1. Dendrogram of PapA variants. Included are the 11 recognized PapA variants (F7-1, F7-2, and F8 to F16), new PapA variants F40 and F48 from the present study, and SMF and MR/P pilins (from *S. marcescens* and *P. mirabilis*, respectively). The unrooted tree was based on (predicted) mature peptides and was constructed using the neighbor-joining method (86). The presumed evolutionary distance between any two members of the tree equals the sum of the lengths of the branches connecting them.

**Nucleotide sequence accession numbers.** The sequences determined in this study have been deposited in GenBank under accession no. Y08931 (F8), Y08927 (F10), Y08923 (F14), Y08929 (F15), and Y08930 (F16).

**RESULTS**

**Derivation set.** After optimization of assay conditions and primer sequences, the F PCR assay yielded the predicted F-specific amplicon(s) for each of the 11 control strains in the derivation set without cross-reactivity between F types. With pooled template DNAs, robust amplicons could be generated simultaneously in a single reaction mixture for all four of the F types represented within each primer pool and products could be readily resolved in agarose gels (Fig. 3).

**First validation set.** Among the 73 strains in the first validation set, many PCR-serotype discrepancies were encountered (Table 1). Thirty-seven (51%) of the strains exhibited at least one such discrepancy. All 37 discrepant strains had an F type detected by PCR that was not expected based on known serological data (i.e., a putative false-positive PCR result); three also had a presumed serological F type that was not detected by PCR (i.e., a putative false-negative PCR result).

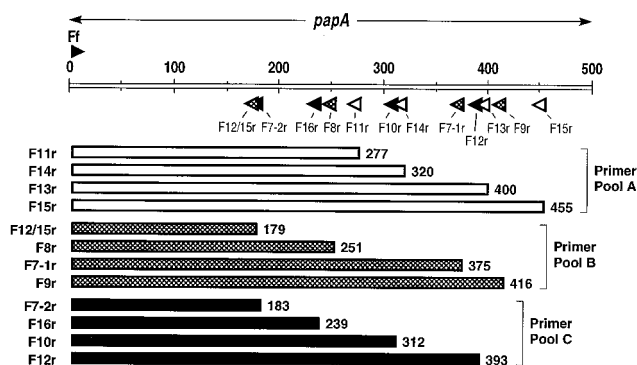


FIG. 2. Distribution of primers and amplicons for multiplex F PCR assay. The universal forward primer Ff (forward-pointing arrowhead above the 5' end of *papA*), in combination with the reverse primers (backward-pointing arrowheads below *papA*), yields a PCR amplicon (wide bars) of specific length with template DNA representing each allele of *papA*. The allele-specific amplicons can be resolved by size in agarose gels when reverse primers are sorted into three pools, i.e., A (white), B (hatched), and C (black).

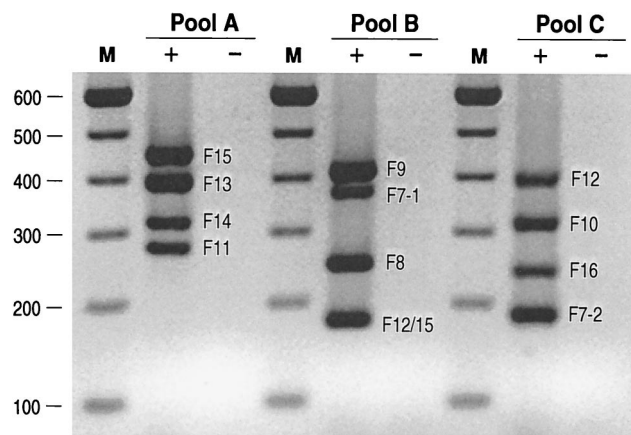


FIG. 3. Gel electrophoresis of multiplex F PCR assay products. PCR was done using primer pools A, B, and C (plus universal forward primer Ff) with pooled template DNA from *pap*-positive control strains representing all 11 known F types (plus lanes) and *pap*-negative control DNA (minus lanes). Amplicons of the expected sizes appear in each of the positive control lanes. No PCR products appear in the negative control lanes. Lanes M, 100-bp ladder. The values on the left are molecular sizes in base pairs.

The putative false-positive PCR results consisted mostly of F10, F14, or F16 PCR products appearing in strains which were not known to be serologically positive for these F types. Thus, although the PCR assay appeared to be highly sensitive for detection of serologically known F types, its specificity was less clear.

Three explanations were considered as possible explanations for the observed PCR-serology discrepancies, including (i) PCR contamination, (ii) nonspecific amplification due to primer cross-reactivity, and (iii) the true presence of (serologically occult) heterologous copies of *papA*. The first possibility, PCR contamination, was ruled out by a series of reproducibility experiments (data not shown). To differentiate between the second and third possibilities, DNA sequences were determined for representative (putative) F10-, F14-, and F16-specific amplicons from control strains 3669, CP9, and IA2, respectively, in which these products were serologically unexpected, and also for the expected F PCR amplicons from these strains (i.e., F9, F13, and F11, respectively). In each instance, the closest match in the sequence data banks for a strain's "extra" F amplicon corresponded to the strain's PCR-detected extra F type rather than with its known serological F type, whereas the closest match for the strain's serologically predicted F amplicon was the corresponding control *papA* sequence (Table 3). Similar direct sequence confirmation that the strain's unexpected putative F10 amplicon represented the authentic F10 *papA* sequence was obtained for F8 and F15 control strains C1254-77 and C1805-79 (data not shown). These results excluded primer cross-reactivity and, instead, strongly suggested the presence of true (serologically unrecognized) heterologous copies of *papA* in the extra-F strains.

As a second proof of the presence of serologically occult *papA* copies, the two *pap* operons from strain CP9 (which gave an unexpected F14 amplicon, as well as the expected F13 amplicon) (Table 1) were isolated as separate cosmid clones for independent analysis. Both cosmid clones expressed P pattern MRHA, evidence that the *pap* operons they contained were functional. One clone was nonhemolytic, contained *papG* allele III by PCR, and yielded the F13 product in the F PCR assay (Table 1). The other was strongly hemolytic, contained *papG* allele I by PCR, and yielded the F14 product in the F

TABLE 3. Confirmation of putative F-specific amplicon sequences

Source strain <sup>a</sup>	Putative F type of amplicon	Unambiguous nucleotides in sequenced amplicon (no.)	BLAST search result					
			Best match			2nd best match		
			% Identity with best match	F type of best match	Accession no. of best match	% Identity with 2nd best match	F type of 2nd best match	Accession no. of 2nd best match
CFT073	F7-1	346	99.4	F7-1	X02921	39.6	F14	Y08928
CFT073	F7-2	158	96.2	F7-2	M12861	67.1	F16	Y08930
C351-82	F8	227	97.8	F8	Y08931	83.7	ECOR 48 <sup>e</sup>	AF051811
C483-82	F9	339	92.6	F9	M68059	14.2	F14	Y08928
3669 <sup>b</sup>	F10 <sup>b</sup>	275	93.1	F10	Y08927	60.7	F13	X61239
IA2	F11	258	95.3	F11	L07420	77.1	ECOR 48 <sup>e</sup>	AF051811
C1979-79	F12	359	98.3	F12	X62157	91.9	F15	Y08929
C1979-79	F12/15 <sup>c</sup>	154	98.7	F12	X62157	98.1	F15	Y08929
CP9	F13	367	94.3	F13	X61239	45.0	F10	Y08927
CP9 <sup>b</sup>	F14 <sup>b</sup>	301	100	F14	Y08928	70.4	F7-1	X02921
C372-82	F15	427	99.7	F15	Y08929	92.7	ECOR 46 <sup>e</sup>	AF051810
C372-82	F12/15 <sup>d</sup>	156	97.4	F12	X62157	96.8	F15	Y08929
IA2 <sup>b</sup>	F16 <sup>b</sup>	220	97.3	F16	Y08930	89.1	F15	Y08929

<sup>a</sup> Strains are as shown in Table 1.

<sup>b</sup> The F10, F14, and F16 amplicons from strains 3669, CP9, and IA2, respectively, represented extra F types. Extra F10 amplicons from F8 and F15 control strains C1254-77 and C1805-79 also were confirmed as representing authentic F10 sequence (data not shown).

<sup>c</sup> From F12.

<sup>d</sup> From F15.

<sup>e</sup> F = antigen type not defined.

PCR assay (Table 1). Thus, the sum of the F PCR results for CP9's two *pap* cosmid clones equaled the F PCR result for the wild-type parent. These findings confirmed the presence in CP9 of two independent and fully functional copies of the *pap* operon, each with its own *papA* allele, as predicted by the F PCR assay.

Further evidence that detection by PCR of multiple F types correlates with the presence of multiple discrete copies of *papA* was provided by F PCR analysis of strain CFT073 and of cosmid clones representing its two *pap* operons, which were known to produce different-size pilins (60); hence, the two *pap* operons were hypothesized to contain distinct *papA* alleles. The wild-type parent was F7-1 plus F7-2 by PCR, and the two cosmid clones were F7-1 and F7-2, respectively. These results were subsequently confirmed by serology (Table 1). (Discovery of the same F profile in strain CFT073 as in F7-1, F7-2 control strain AD110 [Table 1] prompted RAPD fingerprinting of both of these O6:K2:H1 strains, which confirmed a close genomic relationship between them [data not shown].)

The above-described series of experiments suggested that most of the putative false-positive F PCR results actually represented the presence of true *papA* copies which either were not expressed or were expressed but not detected serologically. To differentiate between these two possibilities, selective con-

firmatory reserotyping was done using state-of-the-art methods (including, when indicated, 10× concentrated F10 antiserum) for 33 of the 37 strains from the first validation set for which discrepancies had been noted between the F PCR and historical F serotypes.

Repeat serological testing brought serology into agreement with PCR for 21 (64%) of the 33 previously discrepant strains, confirming that most of the initially observed PCR serology discrepancies represented serological false negatives. The 12 strains which, after confirmatory serotyping, had persisting discrepancies included 11 instances of a PCR-detected extra F type, mostly F10 in an F8-, F9-, or F15-positive control strain, and a single instance of a serological F type (a newly identified F10) not detected by PCR.

To clarify whether these residual PCR-serological discrepancies were due to nonexpression of *papA* versus expression of *papA* without serological detection, the three remaining extra-F strains (C659-81, G1062a, and C826-83) which were F PCR positive only for their extra F type (i.e., F10) and were serologically F negative, i.e., did not have a second serological or PCR-defined F type to confound the analysis (Table 1), were tested for multiple *pap* elements, for other adhesin genes, and for MRHA phenotype (Table 4). Two of the strains (C659-81 and C826-83) were found to have a fragmentary *pap*

TABLE 4. Adhesin genotypes and phenotypes of three control strains positive for F10 by PCR but not by serology

Strain <sup>b</sup>	O:K:H serotype	F serotype		PCR	Adhesin genotype <sup>a</sup>						MRHA pattern	
		Putative <sup>c</sup>	Confirmed <sup>d</sup>		<i>papAH</i>	<i>papC</i>	<i>papEF</i>	<i>papG</i>	<i>papG</i> allele	<i>afa/draBC</i>		<i>sfaS</i>
C659-81	O1:K51:H-	F8	F <sup>-</sup>	F10	+	+	+	-	-	+	-	Non-P
G1062a	O4:K7:Hr	NA <sup>e</sup>	F <sup>-</sup>	F10	+	+	+	+	III	-	-	P
C826-83	O75:K5:H-	F15	F <sup>-</sup>	F10	+	-	-	-	-	-	-	- <sup>f</sup>

<sup>a</sup> As determined by PCR, except for *papAH* in strain C826-83 (PCR negative, probe positive for *papAH*). +, wild type; -, mutant.

<sup>b</sup> Strains C659-81 (alias FS09) and C826-83 (alias FS30) were from the IEKC laboratory. Strain G1062a (alias C892-97) was from the J.R.J. laboratory (Table 1).

<sup>c</sup> Based on historical serotyping results for strains from the IEKC.

<sup>d</sup> Including with 10× concentrated F10 antiserum.

<sup>e</sup> NA, not applicable (strain G1062a was first serotyped during the present study).

<sup>f</sup> -, HA negative.

operon and not to express P-pattern MRHA and, hence, almost certainly did not express an F10 PapA pilin (Table 4). In contrast, strain G1062a had a complete copy of *pap* and expressed P pattern MRHA, consistent with P fimbrial expression. With F10 as its sole *papA* allele, this strain would be expected to express P fimbriae of the F10 type. Thus, this strain's F seronegativity seemed probably to represent a serological false negative.

**Second validation set.** Among strains in the second validation set, although agreement was poor between F PCR and both the historical and the (initial) confirmatory F serotype results, correspondence of PCR to serology improved as serological F profiles were refined in successive rounds of confirmatory serotyping (Table 2). By the third round of serotyping, serology agreed with PCR with respect to the F profile of eight of the nine strains and for 15 (94%) of the 16 F types detected by one or both methods (Table 2). These findings confirmed that conventional F serotyping often substantially underestimates the potential F repertoire of wild-type P-fimbriated strains and that most PCR serotype discrepancies are due to false-negative serological results rather than to PCR false positivity.

**Prevalence of *papA* variants among 75 urosepsis isolates.** With its sensitivity and specificity rigorously confirmed, the F PCR assay was next used to assess the *papA* allele status of 75 blood isolates from patients with urosepsis. Fifty-nine (79%) of the 75 strains were found by PCR to contain one or more of the 11 known *papA* alleles. Of the F PCR-positive strains, 44 had a single F type, 13 had two F types, and 2 had three F types, for a total of 76 detected F types (Fig. 4). Each of the 11 known F types occurred at least once in the population, in decreasing order of prevalence, as follows: F10, 27% (20 strains); F11, 21% (16 strains); F7-2, 17% (13 strains); F16, 11% (8 strains); F14, 8% (6 strains); F8 and F12, 4% (3 strains) each; F7-1, F9, and F13, 3% (2 strains) each; F15, 1% (1 strain). Thus, the three most prevalent F types (F10, F11, and F7-2) accounted for nearly two-thirds (64%) of the F types detected. F7-2, F11, and F16 tended to occur alone (33 [89%] of 37 occurrences), whereas F8, F9, F12, F13, F14, and F15 usually occurred in combination with another F type (15 [88%] of 17 occurrences). The remaining F types (F7-1 and F10) occurred both alone and in combination, each with approximately the same frequency (Fig. 4).

**Phylogenetic distribution of *papA* alleles among urosepsis isolates.** Several of the *papA* alleles exhibited a clear-cut phylogenetic distribution, as evidenced by their strong associations with particular O:K:H serotypes (which usually equate with genetic clonal groups) or carboxylesterase B types (Fig. 4; Table 5). It is noteworthy that although F10 was significantly associated with carboxylesterase B type B2 in general, it was most highly correlated specifically with serogroup O75 (Table 5). Similarly, although F16 was significantly associated with carboxylesterase B type B1 in general, this was due to its independent strong associations with three small subgroups among the B1 strains, i.e., O15:K52:H1, O25:K2:H2, and serogroup O157 (Table 5).

**Associations of *papA* variants with other bacterial traits among urosepsis isolates.** Several of the *papA* variants exhibited statistically significant associations with specific bacterial traits, including other *papA* alleles (Table 6). F7-2 was highly correlated with probe positivity but PCR negativity for the group II capsule synthesis genes *kpsMT*, a genotype which largely equates with K2 capsule (46). F10 and F14 each exhibited multiple associations with other traits, including an association with one another (Table 6). The association of F11 with the K1 variant of *kpsMT* (Table 6) was consistent with its

association with serotypes O1:K1:H7 and O2:K1:H7 (Table 5). In keeping with the association of F16 with serogroup O4 (Table 5), F16 was also associated with the O4 lipopolysaccharide synthesis gene *rfc* (Table 6).

**Discovery of novel *papA* variants.** Two of the urosepsis isolates, U7 and V29, were negative for the 11 known *papA* alleles according to the F PCR assay but still expressed P pattern MRHA and by both dot blot and PCR contained *papAH*, *papC*, *papEF*, and *papG*; hence, they were suspected of harboring novel variants of *papA*. Consequently, the *papAH* amplicons from these two strains were sequenced, translated into peptides, and compared with the 11 known PapA variants. Both new sequences appeared to represent novel *papA* alleles, since each was separated from its nearest neighbor in the PapA tree by a distance greater than that separating the two most closely related of the 11 known PapA variants (i.e., F12 and F15) (Fig. 1); one was actually as distant from its nearest neighbor as any of the known PapA variants was from its own nearest neighbor. The new PapA variant from strain V29 (which was termed F40 because of this strain's laboratory code number) was closely related to the F10 peptide (Fig. 1). PapA from strain U7 (which was termed F48) was an outlier member of the cluster that included the F40, F8, F13, F10, and F11 PapA variants (Fig. 1).

When combined with the universal forward primer (Ff), a reverse primer designed for the new F48 *papA* variant yielded the expected 176-bp amplicon from source strain U7 and did not react with control strains for 11 known F types (data not shown). Reamplification of all 75 urosepsis isolates with the new F48 primer detected a putative F48 *papA* allele in three additional strains, each of which was already known to contain at least one *papA* allele (Fig. 4). Direct sequencing of the putative F48 amplicons from these strains confirmed each as bona fide F48 (data not shown). The new F48 *papA* variant was found to be significantly associated with (but not confined to) serotype O12:K1:H6 (Table 5). It was statistically significantly associated with the F12 *papA* variant, from which it was quite distant at the nucleotide and peptide levels (Fig. 1 and 2), but not with other bacterial traits (Table 6). Because of this association of F48 with F12, the new F48 primer was used to test the F12 control strains. Although the F12 control strains from the first validation set were negative (data not shown), strain AD309 (second validation set), in which F12 had been detected serologically but not by PCR (which was one of only two instances, among all of the control strains, of a putative false-negative PCR result), was positive with the F48 primer (Table 2).

**F10 amplicons from *pap*-negative O75 strains.** Among the urosepsis isolates, the F PCR assay showed all five representatives of serogroup O75 to contain the F10 *papA* allele (Fig. 4). This was surprising, since four of these strains had otherwise tested negative for all *pap* elements by both PCR and (except for one *papAH* probe-positive strain) dot blot assay. Direct sequencing of the putative F10 amplicons from these five strains confirmed that the amplicons represented the authentic F10 *papA* sequence (data not shown). Reexamination of the duplicate dot blots for the three putatively *pap*-negative O75 strains revealed variable faint reactivity with the *papAH* probe, consistent with the presence of a partial copy of *papA*, but no reactivity whatsoever with the probes for *papC*, *papEF*, and *papG* (data not shown). These findings, together with those for the serogroup O75 F15 control strain C826-83 (Table 4) and for several of the other serogroup O75 F8 and F15 control strains (first validation set [Table 1]), suggested that wild-type strains of serogroup O75 commonly contain isolated

Strain	CBT	O:K:H serotype	<i>papAH</i>	F-type	F7-1	F7-2	F8	F9	F10	F11	F12	F13	F14	F15	F16	F40	F48
H16	B2	O1:K1:H7	+	F11						+							
H38	B2	O1:K1:H7	+	F11						+							
V15	B2	O1:K1:H7	+	F11						+							
V23	B2	O1:K1:H7	+	F11						+							
2H18	B2	O1:K1:H7	+	F11						+							
H15	B2	O2:K1:H7	+	F11						+							
H25	B2	O2:K1:H7	+	F11						+							
PM6	B2	O2:K1:H7	+	F11						+							
2P6	B2	O2:K1:H7	+	F11						+							
H35	B2	R:K1:H7	+	F11						+							
V27	B2	O2:K5:H1	+	F10,14					+				+				
H19	B2	O2:K7:H-	+	F10,14					+				+				
V6	B2	O2:K7:H-	+	F10,14					+				+				
V24	B2	O2:K7:H-	+	F7-1,10	+				+								
H1	B2	O2:K7:H1	+	F10,12,15					+		+			+			
V31	NA	O4:K12:H1	+	F10,11					+	+							
PM8	B2	O4:K12:H-	+	F14,16									+			+	
V7	B2	R:K12:H1	+	F10,11,16					+	+							+
H26	B2	O6:K2:H1	+	F7-2		+											
V5	B2	O6:K2:H1	+	F7-2		+											
U3	B2	O6:K2:H1	+	F7-2		+											
PM4	B2	O6:K2:H1	+	F7-2		+											
PM5	B2	O6:K2:H1	+	F7-2		+											
2H6	B2	O6:K2:H1	+	F8,9			+	+									
2H4	B2	O6:K2:H1	+	F7-2		+											
2H24	B2	O6:K2:H1	+	F7-2		+											
V1	B2	O6:H1	+	F7-2		+											
V3	B2	O6:K2:H?	+	F7-2		+											
V16	B2	R:K2:H1	+	F7-2		+											
V19	B2	O6:K5:H-	+	F10,13					+				+				
V22	B2	O6:K5:H-	+	F10,13					+				+				
H3	B2	O6:K53:H7	+	F11						+							
U5	B2	O6:K53:H7	+	F10,14					+				+				
U7	B2	O6:K?H-	+	F48													+
H9	B2	O12:K1:H6	+	F9,12,48				+				+					+
U4	B2	O12:K1:H6	+	F12,48								+					+
2H12	B2	O12:K1:H6	-	-													
PM2	B2	R:K1:H6	-	-													
H7	B2	O16:K1:H-	+	F7-1	+												
2H25	B2	O18ac:K1:H7	+	F10						+							
H5	B2	O18ac:K5:H-	+	F8,10			+		+								
2H21	B2	O21:K100:H5	+	F7-2		+											
V21	B2	O75:K5:H5	-	F10					+								
2H19	B2	O75:K5:H5	+	F8,10			+		+								
V8	B2	O75:K5:H-	-	F10					+								
U8	B2	O75:K100:H5	-*	F10					+								
PM3	B2	O75:K+H-	-	F10					+								
2H11	B2	R:K2:H2	-	-													
2P1	B1	O1:K1:H-	+	F7-2		+											
V14	B1	O2:K5:H-	+	F10,48					+								+
2H7	B1	R:K5:H4	+	F7-2		+											
V20	B1	O7:K1:H-	+	F10					+								
V29	B1	O7:K1:H-	+	F40												+	
2H5	B1	O7:K1:H-	+	F11						+							
PM7	B1	O7:H-	+	F10					+								
H27	B1	O8:K27:H-	+	F11						+							
U6	B1	O8:K27:H-	-*	-													
U1	B1	O8:K44:H9	-	-													
V28	B1	O8:K+H-	-	-													
V9	B1	O9:K34:H-	-	-													
PM9	B1	O9:K34:H-	-	-													
V26	B1	O9:K36:H19	+	F11						+							
V32	B1	O15:O40:K+	+	F14									+				
2H17	B1	O15:K52:H1	+	F16												+	
2P9	B1	O15:K52:H1	+	F16												+	
V11	B1	O17:K53:H18	-	-													
2H16	B1	O25:K2:H2	+	F16												+	
H8	B1	O25:K2:H2	+	F16												+	
H17	B1	O25:K16:H4	-	-													
PM1	B1	O64:H21	-	-													
V10	B1	O77:K54:H18	-	-													
V12	B1	O84:H-	-	-													
2U5	B1	O143:H-	-	-													
H2	B1	O157:H32	+	F16												+	
H18	B1	O157:K52:H45	+	F16												+	



partial copies of the F10 *papA* allele, with or without a separate (complete) *pap* operon containing a different *papA* allele.

## DISCUSSION

In the present study, we developed and rigorously validated a novel PCR-based assay for the 11 recognized variants of *papA*, the major pilin gene of *E. coli* P fimbriae, and then used the assay to assess the prevalence, phylogenetic distribution, and bacteriological associations of the *papA* alleles among 75 *E. coli* isolates from patients with urosepsis. The assay was extremely sensitive and specific, evidence that *papA* sequences are highly conserved within each of the traditionally recognized F serotypes despite the diversity observed among F types. The assay revealed considerable segregation of *papA* alleles according to O:K:H serotype, consistent with vertical transmission within clones, but with exceptions which strongly suggested horizontal transfer of *papA* alleles between lineages. Two novel *papA* variants were identified, one of which was actually more prevalent among the urosepsis isolates than were several of the known *papA* alleles.

The assay's sensitivity approached 100% for detection of serologically evident F types among the diverse control strains in the two validation sets, evidence that the sequences selected for use as F-specific primers are highly conserved within each serological F type. This suggests that the regions targeted by the primers either encode the actual epitopes responsible for F serospecificity (as intended by our primer design strategy) or, if they do not, are nonetheless tightly linked with them. The high degree of homology observed between entire F-specific amplicons and the corresponding F-specific *papA* source sequences (Table 3) suggests that type-specific homology is not limited to just the primer-binding regions but is broadly present throughout *papA*. Full-length sequence analysis of multiple representatives of each F type from phylogenetically distinct backgrounds (8) is needed to more thoroughly evaluate this hypothesis.

The observed patterns of discrepancy between PCR and serology with respect to detection of F types were highly informative. Historically determined serotypes, understandably, did not include certain F types which, at the time of the original serotyping, were uncharacterized or not recognized as corresponding to P fimbriae. This probably accounted for many of the initial discrepancies involving F10, F14, and F16 (F. Scheutz, unpublished data), which constituted the bulk of the extra F types detected by PCR but putatively not by serology (Table 1). Although confirmatory serotyping (which was done initially using standard techniques and reagents and then using intensified methods) did eventually confirm most of the PCR-detectable F types, a small subset remained serologically occult.

At least some of these serologically occult *papA* variants represented nonfunctional copies (or fragments) of *papA*, as documented for one of the F15 control strains (O75:K5:H5) (Table 4) and for four of the serogroup O75 urosepsis isolates (Fig. 4). What proportion of the serologically occult *papA* copies might still be functional under the appropriate conditions (with the possibility of expression, of course, limited to those strains which have an intact *pap* operon either in *cis* or in

*trans* to the serologically inapparent *papA* copy) can only be speculated upon. However, the expression of P pattern MRHA by strain G1062a (Table 4) suggests that at least some of these occult *papA* copies are functional. In any event, the preponderance of evidence indicated that the residual PCR-positive, serotype-negative discrepancies did not represent false-positive PCR results, at least not in the conventional sense of nonspecific primer binding or contamination, but instead represented detection of true *papA* sequences.

Alternative explanations for serological nondetection of documented *papA* alleles, in addition to nonexpression, could include low-level expression (consistent with the demonstrated differences in expression level between different *pap* operons in a single strain) (55) or expression of an antigenically altered pilin. Either hypothesis would be consistent with the improved serological detection of F10 that was observed in the present study with the use of 10× concentrated antiserum.

The PCR assay provided abundant evidence among both the control strains and the urosepsis isolates of clonal segregation of *papA* alleles, as reflected by the distribution of certain *papA* alleles according to O:K:H serotype and carboxylesterase B type. This is consistent with previously reported serological findings (68, 70). Also noted were circumstances suggesting horizontal transfer of *papA* alleles. These included the appearance of diverse *papA* alleles in different members of a single O:K:H serotype, e.g., O2:K7:H-, O4:K12:H1, and O7:K1:H- (Fig. 4), and the local predominance of a particular *papA* allele in evolutionarily distant lineages, e.g., F16 in three non-B2 O:K:H serotypes (O15:K52:H1, O25:K2:H2, and serogroup O157), but also in B2 serotype O4:K12:H1 (Fig. 4; Table 5).

The new assay also facilitated the discovery of novel *papA* variants by revealing the absence of any of the 11 known *papA* alleles in two strains that expressed P pattern MRHA and were *papAH* positive by probe and PCR. Sequencing of the *papAH* amplicons from these two strains revealed a unique *papA* variant in each strain. The subsequent detection of one of the new *papA* variants (F48) in 3 of the 75 urosepsis isolates by using a primer based on the F48 *papA* sequence illustrates how the F PCR assay can be used to discover new *papA* alleles and then screen for them. Discovery of a statistically significant association of the new F48 *papA* variant with F12 prompted retesting of all of the F12 control strains with the new F48 primer, including the strain in which the PCR assay had putatively missed an F12 *papA* allele. Only the latter strain was F48 positive. We are currently investigating the phenotypic correlates of the F48 *papA* allele, including the relationship between F48 and F12 (J. R. Johnson, unpublished data).

Further evidence of the PCR assay's utility as a strain typing tool was provided by the close relationship the assay revealed between two archetypal uropathogenic strains, CFT073 and AD110. CFT073 has been used in studies of pathogenesis (59, 60) and is currently under intensive investigation with respect to the distribution and composition of its pathogenicity islands, including through complete sequencing of its genome by the Fred Blattner laboratory (24, 50; H. L. T. Mobley, personal communication). Venerable strain AD110 was the original source of the first two numbered P fimbrial F types (F7-1 and F7-2) (69) and of recombinant derivatives thereof (95, 97) and has been used in many subsequent studies regarding the reg-

FIG. 4. *papA* alleles among 75 *E. coli* isolates from patients with urosepsis. Strains are sorted according to O:K:H serotype within each carboxylesterase B type (CBT). Results for *papAH* are shown as positive if the blot and PCR assays were both positive, as negative if the blot and PCR assays were both negative, and as negative with an asterisk if the blot assay was positive but the PCR assay was negative. F PCR results are listed in the aggregate and also shown distributed according to individual F types. Serogroup O75 strains V21, V8, and PM3 (shown as *papAH*-negative) exhibited trace reactivity with the *papAH* probe in some blots. Carboxylesterase B data are from reference 39, O:K:H data are from reference 41, and *papAH* data are from reference 46. NA, not assayed.

TABLE 5. Association of F-specific *papA* alleles with serotype and carboxylesterase B type among 75 *E. coli* urosepsis isolates

<i>papA</i> allele (no. of isolates)	Group	No. with <i>papA</i> allele/total (%)		<i>P</i> value
		Group members	Other strains	
F7-2 (13)	O6:K2:H1	10/11 (91)	3/64 (5)	<0.001
F10 (20)	Carboxylesterase B type B2	17/48 (35)	3/27 (11)	0.03
	Serogroup O75	5/5 (100)	15/70 (21)	<0.001
F11 (10)	O1:K1:H7, O2:K1:H7	10/10 (100)	56/65 (9)	<0.001
F12 (3)	O12:K1:H6	2/4 (50)	1/71 (1)	0.006
F13 (2)	O6:K5:H-	2/2 (100)	0/73 (0)	<0.001
F14 (6)	O2:K5:H1, O2:K7:H1	3/5 (60)	3/70 (4)	0.003
F16 (8)	O4:K12:H1	2/3 (67)	6/72 (8)	0.03
	Carboxylesterase B type B1	6/27 (22)	2/48 (4)	0.02
	O15:K52:H1	2/2 (100)	6/73 (8)	0.01
	O25:K2:H2	2/2 (100)	6/73 (8)	0.01
	O157	2/2 (100)	6/73 (8)	0.01
F48 <sup>a</sup>	O12:K1:H6	2/4 (50)	2/71 (3)	0.01

<sup>a</sup> The F48 allele was discovered as part of present study.

ulation of the *pap* operon, P fimbrial structure, and immunological aspects of PapA (12, 13, 55, 79, 80, 101). The finding that CFT073 has the same distinctive F-1, F7-2 *papA* allele profile as does AD110 called attention to these two strains' shared O6:K2:H1 serotype, which suggested a clonal relationship, as subsequently confirmed by RAPD fingerprinting. Prior to the discovery of strain CFT073's *papA* allele profile, neither we nor H. L. T. Mobley (from whose laboratory CFT073 originated) were aware of the considerable similarities between these two archetypal uropathogenic strains (Mobley, personal communication). The discovery of these similarities is significant in that it reveals that there has been an (unrecognized) continuum of investigation over the past 2 decades in different laboratories regarding the pathogenetic mechanisms of what may be essentially the same model uropathogenic strain.

In view of the presence of both the F7-1 and F7-2 *papA* alleles in model pathogens CFT073 and AD110, and occasionally in other O6:K2:H1 strains (68, 70; Johnson, unpublished data), the absence of the F7-1 allele among the O6:K2:H1 urosepsis isolates in the present study is interesting. Almost all of the O6:K2:H1 urosepsis isolates, which constituted the population's single largest clonal group and quite probably equate with virulent clone 4 as described by Korhonen et al. and with clone V of Väisänen-Rhen et al. (53, 90, 92), were positive for F7-2 alone. Whether O6:K2:H1 strains with both F7-1 and F7-2 are simply uncommon enough that by chance alone none was represented in the present study population or whether there are selection factors that favor F7-2-only strains over

F7-1-plus-F7-2 O6:K2:H1 strains as agents of urosepsis remains to be determined. It will be useful to survey O6:K2:H1 (and other) *E. coli* isolates from different extraintestinal syndromes for their *papA* allele content, in part to assess how generally representative are the strains that have emerged as model uropathogens (55, 60, 83, 84, 91) and in part to determine which F types would be most relevant to include in a syndrome-based PapA vaccine (12). The F PCR assay, which was simple, rapid, and easy to interpret, should greatly facilitate future studies of this sort.

This prediction is supported by the results of a "portability" assessment of the F PCR assay, in which 10 wild-type *papA*-positive strains of unknown F status plus strain J96 (an F13 control) were independently F typed in three different laboratories in the United States (J.R.J.), Canada (J. Fairbrother), and France (E. Oswald). The latter two laboratories had only just begun to use the assay when the testing was done. Consistent results were obtained for each strain in each laboratory (Johnson, unpublished data; E. Oswald and J. Fairbrother, personal communication, 1999).

In summary, the F-specific *papA* alleles of extraintestinal *E. coli* were found to be sufficiently conserved within each F type that highly sensitive and specific detection was possible by PCR using a small set of F-specific oligonucleotide primers. The multiplex PCR assay for the 11 known *papA* variants as developed and extensively validated in the present study arguably outperformed conventional F serotyping in accurately detecting *papA* variants. It detected serologically unrecognized *papA*

TABLE 6. Association of F-specific *papA* alleles with other bacterial traits among 75 *E. coli* urosepsis isolates

<i>papA</i> allele (no. of isolates)	Associated trait	No. with <i>papA</i> allele/total (%)		<i>P</i> value
		With trait	Without trait	
F7-2 (13)	<i>kpsMT</i> II probe positive, PCR negative	12/13 (92)	1/62 (2)	<0.001
F10 (20)	<i>cnf-1</i>	8/12 (75)	12/63 (19)	0.001
	K5 <i>kpsMT</i> II	16/25 (64)	4/50 (18)	<0.001
	<i>traT</i>	9/50 (18)	11/25 (44)	0.03
F11 (16)	K1 <i>kpsMT</i> II	12/21 (57)	4/54 (7)	<0.001
F14 (6)	F10	4/20 (25)	2/55 (4)	0.04
	<i>papG</i> allele III	3/5 (60)	3/70 (4)	0.003
	<i>cnf-1</i>	4/12 (33)	2/63 (3)	0.005
	K5 <i>kpsMT</i> II	6/25 (24)	0/50 (0)	<0.001
F16 (8)	<i>rfc</i>	2/3 (67)	6/72 (8)	0.03
F48 <sup>a</sup> (4)	F12	2/3 (67)	2/72 (3)	0.006

<sup>a</sup> The F48 allele was discovered as part of the present study.

copies, demonstrated both clonal segregation and horizontal transfer of *papA* alleles within the *E. coli* population and facilitated the discovery of two novel *papA* variants. The F PCR assay represents a versatile new molecular tool for epidemiological and phylogenetic investigation of the diverse *papA* alleles of extraintestinal pathogenic *E. coli*.

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## AUTHOR'S CORRECTION

### Analysis of the F Antigen-Specific *papA* Alleles of Extraintestinal Pathogenic *Escherichia coli* Using a Novel Multiplex PCR-Based Assay

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Volume 68, no. 3, p. 1587–1599, 2000. Page 1589, Table 1, column 3, line 14 from bottom: “F14” should read “F13.”

Line 15 from bottom: “F13” should read “F14.”

Page 1594, Fig. 4, lines 24, 37, 46, 48, 49, 51, 54, and 73: Strains 2H6, 2H12, U8, 2H11, 2P1, 2H7, 2H5, and 2U5, respectively, should be deleted. We recently discovered through amplification fingerprinting that these eight putative urosepsis isolates that were included in our study of *papA* alleles probably do not represent the actual strains that under the same names were previously tested for O:K:H serotype and carboxylesterase B electrophoretic type. These eight strains are of questionable authenticity. Since there is no ready way to determine whether the previous tested versions or the current versions of these strains represent the authentic clinical isolates, it appears advisable to exclude these strains from future analyses and to disregard results previously obtained for them. Although exclusion of these strains from the published study eliminates several instances of what had appeared to represent probable horizontal transfer of *papA* alleles, this does not substantively alter the conclusions of the study.