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Phylogenetic Analysis and Prevalence of Urosepsis Strains of *Escherichia coli* Bearing Pathogenicity Island-Like Domains

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We characterized 100 *Escherichia coli* urosepsis isolates from adult patients according to host compromise status by means of ribotyping, PCR phylogenetic grouping, and PCR detection of *papG* alleles and the virulence-related genes *sfa/foc, fyuA*, *irp-2*, *aer*, *hly, cnf-1* and *hra*. We also tested these strains for copies of *pap* and *hly* and their direct physical linkage with other virulence genes in an attempt to look for pathogenicity islands (PAIs) described for the archetypal uropathogenic strains J96, CFT073, and 536. Most of the isolates belonged to *E. coli* phylogenetic groups B2 and D and bore *papG* allele II, *aer*, and *fyuA/irp-2*. *papG* allele II-bearing strains were more common in noncompromised patients, while *papG* allele-negative strains were significantly more frequent in compromised patients. Fifteen ribotypes were identified. The three archetypal strains harbored different ribotypes, and only one-third of our urosepsis strains were genetically related to one of the archetypal strains. Three and 18 strains harbored three and two copies of *pap*, respectively, and 5 strains harbored two copies of *hly*. *papGIII* was physically linked to *hly, cnf-1*, and *hra* (reported to be PAI II_{J96}-like genetic elements) in 14% of the strains. The PAI II_{J96}-like domain was inserted within *pheR* tRNA in 11 strains and near *leuX* tRNA in 3 strains. Moreover, the colocalized genes *cnf-1*, *hra*, and *hly* were physically linked to *papGII* in four strains and to no *pap* gene in three strains. *papGII* and *hly* (reported to be PAI I_{CFT073}-like genetic elements) were physically linked in 16 strains, pointing to a PAI I_{CFT073}-like domain. Three strains contained both a PAI II_{J96}-like domain and a PAI I_{CFTO73}-like domain. Forty-two strains harbored *papGII* but not *hly*, in keeping with the presence of a PAI II_{CFT073}-like domain. Only one strain harbored a PAI I_{536}-like domain (*hly* only), and none harbored a PAI I_{J96}-like domain (*papGI* plus *hly*) or a PAI II_{536}-like domain (*papGIII* plus *hly*). This study provides new data on the prevalence and variability of physical genetic linkage between *pap* and certain virulence-associated genes that are consistent with their colocalization on archetypal PAIs.

Escherichia coli is the most frequent cause of gram-negative bacterial extraintestinal infections, such as cystitis, prostatitis, pyelonephritis, bacteremia, and neonatal meningitis, in humans. Several virulence factors enhance the capacity of *E. coli* to cause systemic infections; unlike most commensal *E. coli* strains, extraintestinal isolates possess genes encoding various combinations of adhesins (P and S fimbriae), iron acquisition systems (e.g., aerobactin and yersiniabactin), host defense avoidance mechanisms (capsule or O-specific antigen), and toxins (e.g., hemolysin and cytonecrotizing factor) (13, 14, 17, 42). Genes coding for multiple virulence factors are located together on large blocks of chromosomal DNA known as pathogenicity islands (PAIs) (18).

Recent studies suggest that extraintestinal pathogenic *E. coli* strains belong mostly to phylogenetic group B2 and, to a lesser extent, group D (5, 8, 39). In contrast, commensal *E. coli* strains generally belong to phylogenetic groups A and B1 (12).

In this study, we determined the phylogenetic group, genetic diversity, and virulence gene distribution of 100 well-characterized *E. coli* blood isolates from adults with community-acquired urosepsis, according to host compromise status. We also sought copies of *pap* and *hly* and their direct physical linkage with certain virulence genes, consistent with their colocalization on PAIs described for archetypal uropathogenic *E. coli* strains.

MATERIALS AND METHODS

Bacterial strains. One hundred *E. coli* strains were recovered by blood culture from 100 consecutive adults with community-acquired pyelonephritis and bacteremia between January 1999 and April 2001 in the Paris, France, region. A spontaneous nonhemolytic mutant of one of these strains (P89-M1) was also studied.

Pyelonephritis strain CFT073 from Baltimore, Md. (kindly provided by H. L. T. Mobley, University of Maryland) (32); pyelonephritis strain 536 from Wurzburg, Germany (kindly provided by J. Hacker) (7); and pyelonephritis strain J96 from Seattle, Wash. (kindly provided by J. Hacker) (6) were used for comparison.

Clinical data. Community-acquired bacteremia was defined as a bacteremic episode in which, at the time when the index blood sample was taken for culture, the patient was not hospitalized or had been hospitalized for less than 48 h and had not been hospitalized during the previous 28 days. Diagnostic criteria for acute pyelonephritis were dysuria, temperature of $\geq$38.5°C, leukocyturia of $>10^5$/ml, *E. coli* level of $\geq 10^5$ CFU/ml in midstream urine, and no other identifiable source of infection. The following data were recorded from the patients' files: age, gender, and host compromise status (i.e., underlying medical conditions such as diabetes mellitus, cancer, immunosuppression, or uremia or underlying urological conditions such as preexisting urinary tract abnormalities, urinary tract instrumentation, or pregnancy).

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TABLE 1. Oligonucleotide primers used to amplify virulence-associated genes

Primer designation	Primer sequence	Target	Size of PCR product (bp)	Reference
chuA.1	5′-GACGAACCAACGGTCAGGAT-3′	*chuA*	279	(9)
chuA.2	5′-TGCCGCCAGTACCAAAGACA-3′			(9)
yjaA.1	5′-TGAAGTGTCAGGAGACGCTG-3′	*yjaA*	211	(9)
yjaA.2	5′-ATGGAGAATGCGTTCCTCAAC-3′			(9)
TspE4C2.1	5′-GAGTAATGTCGGGGCATTCA-3′	TspE4.C2	152	(9)
TspE4C2.2	5′-CGCGCCAACAAAGTATTACG-3′			(9)
papC.1	5′-GACGGCTGTACTGCAGGGTGTGGCG-3′	*papC*	328	(33)
papC.2	5′-ATATCCTTTCTGCAGGGATGCAATA-3′			(33)
papG.I.1	5′-TCGTGCTCAGGTCCGGAATTT-3′	*papGI*	461	(23)
papG.I.2	5′-TGGCATCCCCCAACATTATCG-3′			(23)
papG.II.1	5′-GGGATGAGCGGGCCTTTGAT-3′	*papGII*	190	(23)
papG.II.2	5′-CGGGCCCCCAAGTAACTCG-3′			(23)
papG.III.1	5′-GGCCTGCAATGGATTTACCTGG-3′	*papGIII*	258	(23)
papG.III.2	5′-CCACCAAATGACCATGCCAGAC-3′			(23)
papG.II/III.1	5′-GACTCTTTCTGTGTCTTGCG-3′	*papGII* + *papGIII*	254	This study
papG.II/III.2	5′-GAACCAGATAGTACTCCTGG-3′			This study
sfa.1	5′-CTCCGGAGAACTGGGTGCATCTTAC-3′	*sfa*/*foc*	410	(33)
sfa.2	5′-CGGAGGAGTAATTACAAACCTGGCA-3′			(33)
hly.1	5′-AGGTTCTTGGGCATGTATCCT-3′	*hlyC*	556	(5)
hly.2	5′-TTGCTTTGCAGACTGCAGTGT-3′			(5)
cnf1.1	5′-CAGTGACCGGATCTCCGTTAT-3′	*cnf-1*	230	(38)
cnf1.2	5′-CGTGTAATTCTTCTGTACTTCC-3′			(38)
aer.1	5′-AAACCTGGTTTACGCAACTGT-3′	*aer* (*iucC*)	269	(5)
aer.2	5′-ACCCGTCTGCAAATCATGGAT-3′			(5)
fyuA.1	5′-TGATTAACCCCGCGACGGGAA-3′	*fyuA*	780	(31)
fyuA.2	5′-CGCAGTAGGCACGATGTTGTA-3′			(44)
irp2.1	5′-AAGGATTCGCTGTTACCGGAC-3′	*irp-2*	280	(44)
irp2.2	5′-TCGTCGGGCAGCGTTTCTTCT-3′			(44)
hra.1	5′-CAGAAAACAACCGGTATCAG-3′	*hra*	260	This study
hra.2	5′-ACCAAGCATGATGTCATGAC-3′			This study
pheR.1	5′-GCCGCAATCTTAAGCAGTTG-3′	*pheR*	350	This study
pheR.2	5′-GCACGACATTTCACGTCAGT-3′			This study
yjgB.1	5′-ACCTTGCTCGCAGTTGATCT-3′	*leuX (yjgB)*		This study

Detection of virulence determinants by PCR. All isolates were tested for seven putative virulence factor genes characteristic of extraintestinal pathogenic *E. coli* (*chuA*, outer membrane heme receptor; *pap*, P fimbriae; *sfa*, S fimbriae; *hly*, hemolysin; *cnf-1*, cytotoxic necrotizing factor; *aer*, iron uptake system; and *fyuA*/*irp-2*, iron uptake system). In addition, the 21 *cnf-1*-positive isolates were screened for *hra* (heat-resistant agglutinin) (45). The PCR was carried out in a 20-μl volume with 2 μl of 10× buffer (ATGC Biotechnologie, Noisy-le-Grand, France), 20 pmol of each primer, a 200 μM concentration of each deoxynucleoside triphosphate, 2.5 U of *Taq* polymerase (ATGC Biotechnologie), and 3 μl of bacterial lysate. PCR was performed with a Perkin-Elmer GeneAmp 9600 thermal cycler with MicroAm tubes under the following conditions: denaturation for 5 min at 94°C; 30 cycles of 10 s at 94°C, 20 s at 55°C, and 30 s at 72°C; and a final extension step for 7 min at 72°C. The primers used for PCR (Table 1) were chosen from previously published sequences or were designed for this study (5, 9, 23, 31, 33, 38, 44).

PCR phylogenetic grouping. The phylogenetic group was determined by using a previously described PCR-based method (9). Briefly, two-step triplex PCR was performed directly on 3 μl of bacterial lysate with the primer pairs chuA.1-chuA.2, yjaA.1-yjaA.2, and TspE4C2.1-TspE4C2.2 (Table 1). The PCR steps were as follows: denaturation for 4 min at 94°C, 30 cycles of 5 s at 94°C and 10 s at 59°C, and a final extension step for 5 min at 72°C.

Ribotyping. Total *E. coli* DNA was prepared as previously described (3, 4) and then digested with *Hin*dIII and subjected to Southern blotting with *E. coli* 16S rRNA plus 23S rRNA as the probe (2). The probe was labeled by random oligonucleotide priming with a mixture of hexanucleotides (Amersham Pharmacia Biotech, Saclay, France) and cloned Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gibco BRL, Cergy Pontoise, France) in the presence of 0.35 mM digoxigenin-11-deoxyuridine-5′ triphosphate (Roche, Meylan, France). Chemiluminescence was detected as previously reported (3).

PFGE and Southern blot hybridization. Pulsed-field gel electrophoresis (PFGE) was used to determine the physical locations of genes representative of PAI II_{J96}-like genetic elements (*papGIII*, *hly*, *cnf-1*, and *hra*) (45) and of PAI I_{CFTO73}-like genetic elements (*papGII* and *hly*) (32). Thirty-four *hly*-positive strains and the three archetypal strains J96, CFT073, and 536 were subjected to PFGE as previously described (15). In brief, bacterial cells were embedded in agarose, lysed with detergent and proteinase K (Sigma-Aldrich, St Quentin Fallavier, France), and digested with *Not*I (Roche). PFGE digests were transferred to nylon membranes (Amersham Pharmacia Biotech) and hybridized with digoxigenin-labeled probes generated from the primers specific for each gene (Table 1) as recommended by the manufacturer (Roche).

Determination of the copy numbers of virulence genes. Southern blots used for ribotyping were also used to determine the *papG*, *aer*, and *hly* copy numbers by hybridization with digoxigenin-labeled probes. Strains harboring more than one copy of these genes were subjected to another round of Southern blotting after digestion with *Eco*RI, followed by hybridization with the same probes.

Localization of the PAI II_{J96}-like junctions on the K-12 chromosome map. Long-range PCR was performed with the Expand Long Template PCR system (Roche) to amplify the DNA region between *pheR* or *leuX* and *hra*, using the same primers as for standard PCR (Table 1). The PCR was carried out according to the manufacturer's instructions in a 30-μl volume with 3 μl of buffer 3, 12 pmol of each primer, a 500 μM concentration of each deoxynucleoside triphosphate, 2.5 U of *Taq* mix, and 200 ng of genomic DNA.

Capsular typing. K1 antigen was detected with an antiserum to a *Neisseria meningitidis* group B strain (11).

Statistical analysis. Fisher's exact test was used. P values of <0.05 were considered statistically significant.

RESULTS

Patients' characteristics. Eighty-one of the 100 patients were women. The median age was 66 years (range, 19 to 99 years). The median ages of the male and female patients were

TABLE 2. Host characteristics according to *papG* alleles and phylogenetic group

Associated host characteristic (*n*)	No. (%) of strains								
	With *papG* allele:			*papC* positive *papG* negative (*n* = 5)	*pap* negative (*n* = 22)	From phylogenetic group:			
	II only (*n* = 59)	*III* only (*n* = 5)	*II* + *III* (*n* = 9)			B2 (*n* = 61)	D (*n* = 27)	A (*n* = 11)	B1 (*n* = 1)
Noncompromise (41)	32[a] (78)	2 (5)	2 (5)	1 (2)	4[b] (10)	23 (56)	15 (37)	3 (7)	0 (0)
Any compromise (59)	27 (46)	3 (5)	7 (12)	4 (7)	18 (30)	38 (64)	12 (20)	8 (14)	1 (2)
Diabetes (24)	16	0	1	2	5	15	6	3	0
Cancer (6)	2	0	0	1	3	4	1	1	0
Immunosuppression (11)	5	0	0	1	5	8	1	2	0
Uremia (14)	6	1	3	0	4	10	3	1	0
Any urological compromise (8)	1	1	1	1	4	4	1	2	1
Urinary tract instrumentation (5)	3	1	0	0	1	4	1	0	0
Pregnancy (6)	2	0	3	0	1	4	2	0	0

[a] $P = 0.002$ versus any host compromise.
[b] $P = 0.02$ versus any host compromise.

67 and 64 years, respectively. Fifty-three percent of the patients were over 65 (81% of males and 47% of females). Forty-one patients were noncompromised, and 59 had one or more host compromise factors (Table 2). The median age was 58 years in the noncompromised group and 66 years in the compromised group.

Phylogenetic analysis and ribotyping. Sixty-one, 27, 11, and 1% of the isolates belonged to phylogenetic groups B2, D, A, and B1, respectively (Tables 2 and 3). Fifteen ribotypes (I to XV) were identified among the 100 isolates. The numbers of ribotypes in groups B2, D, A, and B1 were 6, 5, 3, and 1, respectively. However, four ribotypes (I, $n = 20$; II, $n = 19$; III, $n = 12$; and IV, $n = 12$) accounted for 63% of the isolates. Five ribotypes were represented by seven or more isolates, and two ribotypes were represented by a single isolate (Fig. 1; Table 4). Representative ribotypes are shown in Fig. 1. The archetypal strains CFT073, 536, and J96 harbored ribotypes I, III, and XI, respectively, and could thus be considered genetically related to, respectively, 20, 12, and 2 of our strains.

Prevalence of virulence factors. *papC* was found in 78 isolates, 73 of which harbored *papGII* and/or *papGIII*. The most prevalent *papG* allele was allele II (68%) (Tables 3 and 4). Allele III was present in only 14% of strains, and none of the strains contained *papG* allele I. *fyuA* and *irp-2* were detected in 92% of the isolates. *sfa*/*foc*, *aer*, *hly*, and *cnf-1* were found in 2,680, 34, and 21% of the isolates, respectively. Eighteen isolates had two copies of *pap*, and two strains had three copies. Five strains had two copies of *hly*, and five strains had two copies of *aer* (Table 4). *papG* allele III, *sfa*/*foc*, and *cnf-1* were restricted to phylogenetic group B2 strains. K1 antigen was detected in 20 isolates, of which 18 belonged to phylogenetic group B2 and 13 belonged to ribotype II.

Prevalence and physical location of virulence gene combinations. As combinations of some virulence genes with *pap* and/or *hly* may suggest the presence of PAIs described for archetypal strains (18, 19), we examined various combinations of *papG* and virulence factors. PFGE was applied to strains with putative PAI II_{J96}-like domains (*papGIII*, *hly*, *cnf-1*, *hra* positive) and putative PAI I_{CFT073}-like domain (*papGII*, *hly* positive). Southern blotting of PFGE gels showed that *hly* was physically linked to *cnf-1* and *hra* in 21 strains. These three genes were linked to *papGIII* in 14 strains and to *papGII* in 4 strains (P28, P52, P77, and P83) and were found alone in 3 strains (P50, P57, and P167) (Table 4). The presence of colocalized PAI II_{J96}-like genetic elements in 14 strains suggested the presence of a PAI similar to PAI II_{J96}. Interestingly, one strain (P100) bore two copies of the physically linked combination of *papGIII*, *hly*, *cnf-1*, and *hra* (Fig. 2). *hly* was physically linked to *papGII* alone in 16 strains. Three of these latter strains also harbored another copy of *hly*, linked to *cnf-1* and *hra*. The smallest *Not*I DNA fragment containing *papGII* and *hly* was approximately 200 kb long, and those containing *hly*, *cnf-1*, and *hra* were about 280 kb long (data not shown).

During subculture, a nonhemolytic colony of P89 occurred spontaneously. This mutant, designated P89-M1, was further investigated. PFGE of DNA digested with *Not*I showed that P89-M1 had a DNA fragment approximately 120 kb shorter than that of the wild-type strain (700 versus 580 kb) (Fig. 2). PFGE Southern blot hybridization with *papGII*/*GIII*, *hly*, *cnf-1*, and *hra* probes showed that all of these genes (physically linked

TABLE 3. Distribution of virulence factors in *E. coli* urosepsis strains according to phylogenetic group

Group (*n*)	No. (%) with:								
	papC	*papG* Class II	*papG* Class III	*papG* Class II + III	*sfa*/*foc*	*aer*	*hly*	*cnf-1*	*fyuA*/*irp-2*
Total (100)	78	59	5	9	26	80	34	21	92
A (11)	5 (45)	1 (9)	0	0	0	6 (55)	0	0	11 (100)
B1 (1)	0	0	0	0	0	0	0	0	0
D (27)	21 (78)	20 (74)	0	0	0	26 (96)	1 (37)	0	20 (74)
B2 (61)	52 (85)	38 (62)	5 (8)	9 (15)	26 (43)	48 (79)	33 (54)	21 (34)	61 (100)

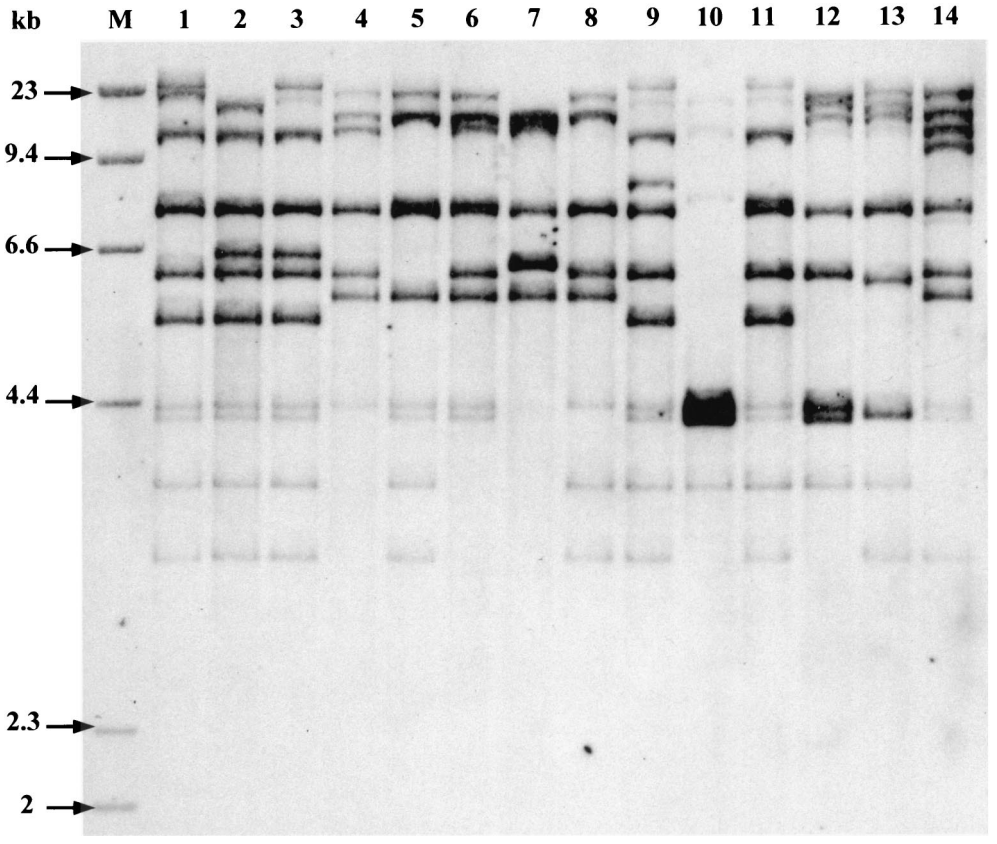

FIG. 1. Representative ribotypes after *Hin*dIII digestion of the 100 urosepsis strains. Lane 1, ribotype I (20 strains, group B2); lane 2, ribotype II (19 strains, group B2); lane 3, ribotype III (12 strains, group B2); lane 4, ribotype IV (12 strains, group D); lane 5, ribotype V (7 strains, group D); lane 6, ribotype VI (6 strains, group A); lane 7, ribotype VII (4 strains, group A); lane 8, ribotype VIII (4 strains, group D); lane 9, ribotype IX (4 strains, group B2); lane 10, ribotype X (4 strains, group B2); lane 11, ribotype XI (2 strains, group B2); lane 12, ribotype XII (2 strains, group D); lane 13, ribotype XIII (2 strains, group D); lane 14, ribotype XIV (1 strain, group B1); lane M, molecular size marker.

on the 700-kb *Not*I DNA restriction fragment) were absent from the 580-kb DNA fragment (Fig. 2). This demonstrated that the genes characteristic of PAI II_{J96} were clustered on a DNA fragment of approximately 120 kb on the P89 chromosome and were spontaneously deleted en bloc. These characteristics are in accordance with those described for PAI II_{J96}, demonstrating that strain P89 harbors a PAI similar to PAI II_{J96} (6, 45).

To determine whether the PAI II_{J96}-like domain found in 14 strains was inserted within the *pheR* tRNA gene, as described for strain J96, PCR was performed with primers homologous to the flanking sequence of the *pheR* tRNA gene (Table 1). DNAs from 11 of these 14 strains could not be amplified, suggesting that their *pheR* tRNA genes were interrupted. To confirm that this interruption was caused by the insertion of a PAI II_{J96}-like domain, we used a long-range PCR approach. Amplification with primers chosen within the sequence of *hra* (hra.1) (a gene located at the end of the PAI) (45) and the flanking region of *pheR* tRNA (pheR.1) yielded a fragment of 5.2 kb with strain J96. Thus, long-range PCR was applied to each of the 11 strains, and 6 and 5 of them, respectively, yielded fragments of 5.2 and 7.5 kb (data not shown). Regarding the three strains (P76, P53, and P97) in which the *pheR* tRNA gene was not interrupted, we speculated that the leucine X tRNA region was the other insertion site of the PAI II_{J96}-like domain. This assumption was based on work by Hacker et al. (18), who described a PAI on strain 536 which may resemble PAI II_{J96} (*papGIII* positive, *hly* positive, and *cnf-1* negative but having the *cnf-1* flanking sequence) and is inserted in the vicinity of the leucine X tRNA region. As *hra* was also present in strain 536 and physically linked to *papGIII* and *hly* (see below) (Fig. 2), long-range PCR between *hra* and the leucine X region (*yjgB* gene) (Table 1) was performed with this strain and yielded a fragment of 8 kb. Long-range PCR was also applied to the three strains (P76, P53, and P97) with positive *pheR* amplification and gave fragments of 8, 4.5, and 4.5 kb, respectively. With strain P100, harboring two PAI II_{J96}-like domains, long-range PCR (hra.1-pheR.1 and hra.1-yjgB.1) gave fragments of 5.2 and 4.5 kb, respectively. Thus, four strains had a PAI II_{J96}-like domain near the *leuX* tRNA gene.

Finally, as the colocalized genes *hra*, *cnf-1*, and *hly*, found in seven strains lacking the *papGIII* allele (strains P50, P57, P83, P167, P28, P52, and P77), may correspond to a modified PAI II_{J96}-like domain, we analyzed these strains in the same way. We found that the *pheR* tRNA genes were interrupted in all seven strains. Long-range PCR (hra.1-pheR.1) yielded products of 5.2 and 7.5 kb with five and two strains, respectively (Table 4).

TABLE 4. Characteristics of the 100 *E. coli* urosepsis isolates and of the three archetypal strains[a]

Strains N°	Ribotype	*pap* C	*pap* G alleles II	*pap* G alleles III	*hly*	*cnf* 1	*hra*	*sfa/foc*	*aer*	*fyu* A	*irp* 2	K1 antigen	phylogenetic group
CFT 073	I	+	+ +	-	+	-	-	+	+	+	+	-	B2
E 536	III	+	-	+	+ +	-	+ c	+	-	+	+	-	B2
J 96	XI	+	-	+	+ +	+	+ a	+	-	+	+	-	B2
P1	I	+	+	+	+	+	+ b	+	+	+	+	-	B2
P9	I	+	+	+	+	+	+ a	+	+	+	+	-	B2
P27	I	+	+	+	+ +	+	+ a	+	+	+	+	-	B2
P36	I	+	+	+	+	+	+ b	+	+	+	+	-	B2
P60	I	+	+	+	+	+	+ b	+	+	+	+	-	B2
P84	I	+	+	+	+ +	+	+ a	-	+	+	+	-	B2
P89	I	+	+	+	+	+	+ a	+	+	+	+	-	B2
P166	I	+	+	+	+	+	+ b	+	+	+	+	-	B2
P169	I	+	+	+	+	+	+ b	+	+	+	+	-	B2
P28	I	+	+ +	-	+	+	+ a	-	+	+	+	-	B2
P7	I	+	+	-	+	-	nd	+	+	+	+	-	B2
P34	I	+	+	-	+	-	nd	+	+	+	+	-	B2
P35	I	+	+	-	+	-	nd	+	+	+	+	-	B2
P48	I	+	+	-	+	-	nd	-	+	+	+	-	B2
P75	I	+	+	-	-	-	nd	+	+	+	+	-	B2
P50	I	-	-	-	+	+	+ a	+	+	+	+	-	B2
P57	I	-	-	-	+	+	+ a	+	-	+	+	-	B2
P167	I	-	-	-	+	+	+ b	+	-	+	+	-	B2
P15	I	-	-	-	-	-	nd	+	+	+	+	+	B2
P73	I	-	-	-	-	-	nd	-	-	+	+	-	B2
P18	II	+	+	-	-	-	nd	-	-	+	+	-	B2
P86	II	+	+	-	-	-	nd	-	-	+	+	+	B2
P93	II	+	+	-	-	-	nd	-	-	+	+	+	B2
P80	II	+	+	-	-	-	nd	+	+	+	+	+	B2
P2	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P5	II	+	+	-	-	-	nd	-	+	+	+	-	B2
P12	II	+	+	-	-	-	nd	-	+	+	+	-	B2
P16	II	+	+	-	-	-	nd	-	+	+	+	-	B2
P20	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P23	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P24	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P29	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P31	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P40	II	+	+	-	-	-	nd	-	+	+	+	-	B2
P49	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P55	II	+	+	-	-	-	nd	-	+	+	+	-	B2
P59	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P91	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P165	II	+	+	-	-	-	nd	-	+	+	+	+	B2
P76	III	+	-	+ +	+	+	+ c	+	-	+	+	-	B2
P52	III	+	+ +	-	+ +	+	+ a	+	+	+	+	-	B2
P77	III	+	+ +	-	+	+	+ b	+	+	+	+	-	B2
P3	III	+	+	-	+	-	nd	-	+	+	+	-	B2
P17	III	+	+	-	+	-	nd	-	+	+	+	-	B2
P47	III	+	+	-	+	-	nd	-	+	+	+	-	B2
P79	III	+	+	-	+	-	nd	-	+ +	+	+	-	B2
P163	III	+	+ + +	-	+	-	nd	-	+	+	+	+	B2
P43	III	+	+ +	-	-	-	nd	+	+	+	+	-	B2
P66	III	+	+	-	-	-	nd	-	+	+	+	-	B2
P63	III	-	-	-	-	-	nd	-	+	+	+	-	B2
P164	III	-	-	-	-	-	nd	-	+	+	+	-	B2

TABLE 4—*Continued*

P14	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P32	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P37	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P44	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P71	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P81	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P94	IV	+	+	-	-	-	nd	-	+	+	+	-	D
P99	IV	+	+	-	-	-	nd	-	+	-	-	-	D
P85	IV	+	-	-	-	-	nd	-	+	-	-	-	D
P33	IV	-	-	-	-	-	nd	-	+	-	-	-	D
P39	IV	-	-	-	-	-	nd	-	+	-	-	-	D
P162	IV	-	-	-	-	-	nd	-	-	-	-	-	D
P98	V	+	+	-	+	-	nd	-	+ +	+	+	-	D
P10	V	+	+	-	-	-	nd	-	+	+	+	-	D
P19	V	+	+	-	-	-	nd	-	+	+	+	-	D
P42	V	+	+	-	-	-	nd	-	+	+	+	-	D
P46	V	+	+	-	-	-	nd	-	+	+	+	-	D
P92	V	+	+	-	-	-	nd	-	+	+	+	-	D
P22	V	-	-	-	-	-	nd	-	+	+	+	-	D
P11	VI	+	-	-	-	-	nd	-	+	+	+	-	A
P38	VI	+	-	-	-	-	nd	-	+	+	+	-	A
P56	VI	+	-	-	-	-	nd	-	+	+	+	-	A
P72	VI	+	-	-	-	-	nd	-	-	+	+	-	A
P26	VI	-	-	-	-	-	nd	-	+	+	+	-	A
P78	VI	-	-	-	-	-	nd	-	+	+	+	-	A
P61	VII	+	+	-	-	-	nd	-	+	+	+	-	A
P90	VII	-	-	-	-	-	nd	-	-	+	+	-	A
P96	VII	-	-	-	-	-	nd	-	-	+	+	-	A
P161	VII	-	-	-	-	-	nd	-	-	+	+	-	A
P45	VIII	+	+	-	-	-	nd	-	+	+	+	-	D
P54	VIII	+	+	-	-	-	nd	-	+	+	+	-	D
P87	VIII	+	+	-	-	-	nd	-	+	+	+	-	D
P168	VIII	+	+	-	-	-	nd	-	+ +	+	+	-	D
P53	IX	+	-	+	+	+	+ d	+	-	+	+	-	B2
P97	IX	+	-	+	+	+	+ d	+	-	+	+	+	B2
P100	IX	+	-	+ +	+ +	+ +	++ a,d	+	-	+	+	+	B2
P70	IX	+	+ + +	-	+	-	nd	-	+	+	+	+	B2
P6	X	+	+ +	-	+	-	nd	-	+ +	+	+	-	B2
P65	X	+	+	-	+	-	nd	-	+ +	+	+	-	B2
P25	X	-	-	-	-	-	nd	-	-	+	+	-	B2
P58	X	-	-	-	-	-	nd	-	+	+	+	-	B2
P74	XI	+	-	+ +	+ +	+	+ a	+	-	+	+	-	B2
P83	XI	+	+ +	-	+	+	+ a	+	-	+	+	-	B2
P13	XII	+	+	-	-	-	nd	-	+	+	+	+	D
P21	XII	-	-	-	-	-	nd	-	+	-	-	-	D
P88	XIII	+	+	-	-	-	nd	-	+	+	+	+	D
P68	XIII	-	-	-	-	-	nd	-	+	-	-	-	D
P82	XIV	-	-	-	-	-	nd	-	-	-	-	-	B1
P95	XV	-	-	-	-	-	nd	-	-	+	+	-	A

[a] Physically linked genes on PFGE are represented in the same color (only *hly*-positive strains were tested). nd, not done; number of (+), number of copies of the gene. a, PCR *hra*/*pheR* fragment size, 5.2 kb; b, PCR *hra*/*pheR* fragment size, 7.5 kb; c, PCR *hra*/*leuX* fragment size, 8 kb; d, PCR *hra*/*leuX* fragment size, 4.5 kb.

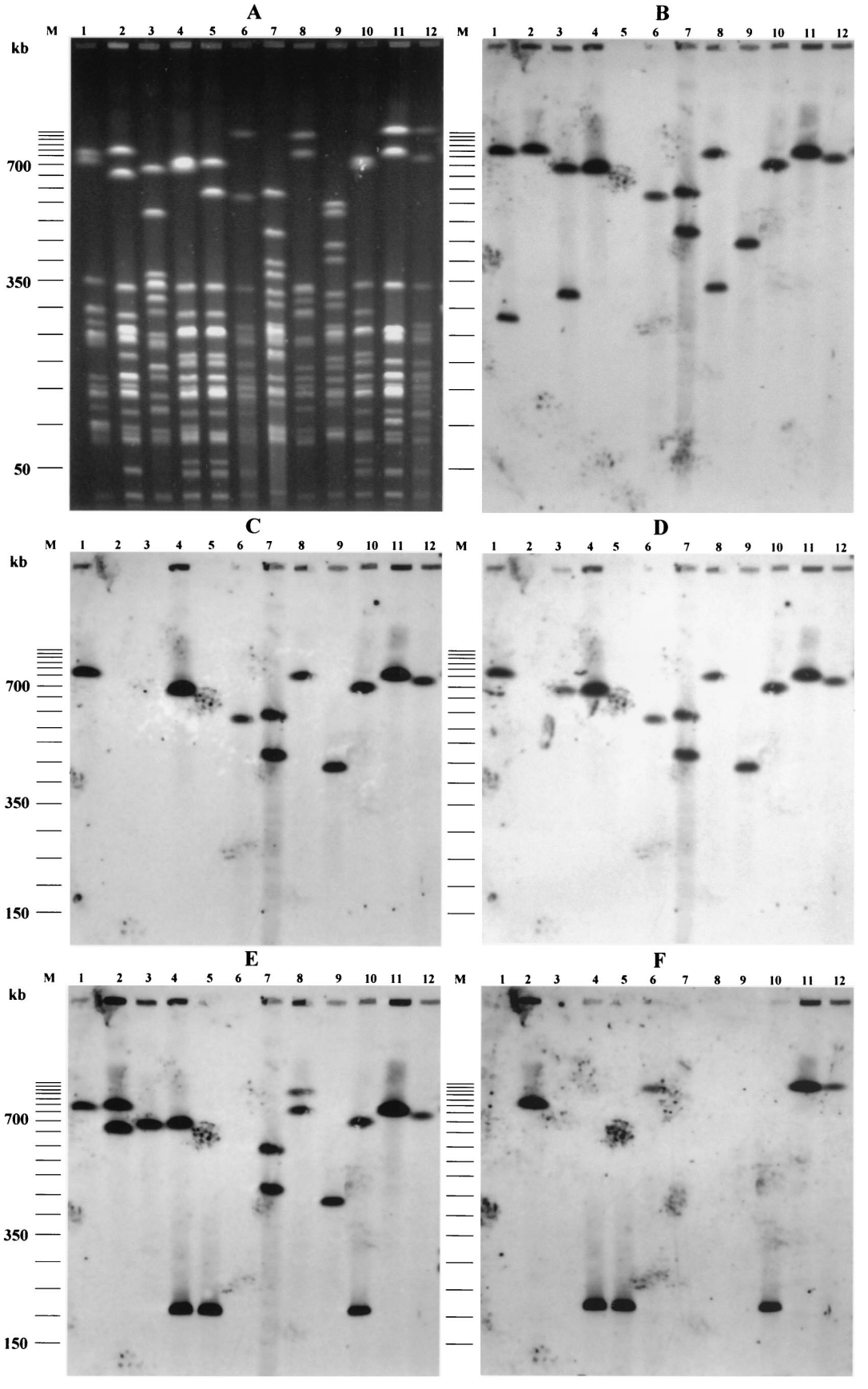

FIG. 2. PFGE patterns of *Not*I-digested genomic DNAs (A) and Southern hybridization with DNA probes specific for *hly* (B), *cnf-1* (C), *hra* (D), *papGII/III* (E), and *aer* (F) of archetypal and representative strains. Lanes 1, J96; lanes 2, CFT073; lanes 3, 536; lanes 4, P89; lanes 5, P89-M1; lanes 6, P50; lanes 7, P100; lanes 8, P74; lanes 9, P53; lanes 10, P9; lanes 11, P166; lanes 12, P28; lanes M, molecular size marker (50-kb DNA ladder).

Using the Blattner website (www.genome.wisc.edu/html/upec.html), which gives the sequence of CFT073 (16, 32), we found that *hly* and *papGII* were present on a 124-kb fragment inserted between *pheV* tRNA and *yghD* genes on the K-12 chromosome map and that the *aer* operon was present in this fragment. Thus, as *aer* was more frequent in *papGII*-positive isolates (64 of 68 [93%]) than in *papGII*-negative isolates (17 of 32 [53%]) ($P < 0.01$) (Table 4), we investigated the physical linkage of *aer* and *papGII* in the strains subjected to PFGE. Among the 26 strains harboring *aer*, *pap* and *aer* were physically linked in 17. Moreover, *papGII, hly*, and *aer* were physically linked in 15 of these strains. The other two strains harbored *papGII* and *aer*, but not *hly*, colocalized on the same DNA macrorestriction fragment of 210 kb (Fig. 2). Finally, in only one isolate (P74) *hly* was not physically linked to *pap*. Interestingly, 42 isolates harbored *papGII* without *hly* or *cnf-1*, but 39 of these isolates also harbored *aer* (Table 4).

PFGE Southern blots of representative strains carrying the *hly* and *cnf-1* genes (P9, P28, P50, P53, P74, P89, P100, and P166), as well as mutant P89-M1 and the archetypal strains J96, CFT073, and 536, are shown in Fig. 2. As expected, *hly* was coupled to *papGIII*, *cnf-1*, and *hra* on a 760-kb *Not*I DNA fragment in strain J96 and was present alone on a 290-kb *Not*I DNA fragment. Strain CFT073 harbored two *papGII* alleles, one of which was physically linked to *hly* and *aer* on an 800-kb *Not*I DNA fragment. Interestingly, strain 536 harbored a *papGIII* gene that was physically linked, on a 680-kb *Not*I DNA fragment, with *hly* (as expected) and also *hra* (Fig. 2).

Phylogenetic group versus host characteristics. The phylogenetic group distribution did not differ significantly between compromised and noncompromised patients (Table 2).

***papG* alleles versus host characteristics.** Strains bearing only allele II were more common in noncompromised patients than in compromised patients ($P = 0.002$) (Table 2). In contrast, *papG*-negative strains were significantly more frequent among compromised patients ($P = 0.005$) (Table 2).

DISCUSSION

We used molecular methods to characterize a set of community-acquired urosepsis *E. coli* isolates obtained in France and to compare these strains with archetypal uropathogenic strains. The diversity of the isolates was shown by the presence of 15 ribotypes, although a small number of ribotypes accounted for most of the isolates. Such oligoclonality in extraintestinal *E. coli* has previously been reported (20, 35, 36). One-third of isolates were accounted for by the three ribotypes to which the three archetypal strains belonged; the most frequent, ribotype I ($n = 20$), was harbored by strain CFT073, which is the only urosepsis strain among the three archetypal strains (32). However, isolates belonging to the second most frequent ribotype (ribotype II) were genetically unrelated to any of the three archetypal strains and were homogeneous with respect to their virulence factor profiles and genetic background. Indeed, all isolates belonging to ribotype II ($n = 19$) carried *papGII*, *fyuA*, and *irp-2* and lacked both *hly* and *cnf-1*, suggesting close genetic relatedness.

Most uropathogenic *E. coli* strains are reported to belong to phylogenetic group B2 (20, 25, 40), but the distribution of community-acquired urosepsis isolates among the other phylogenetic groups (A, B1, and D) has not previously been assessed. Using a triplex PCR method (9), we found that most of our isolates belonged to group B2 ($n = 61$) and, to a lesser extent, group D ($n = 27$). The fourth most frequent ribotype (ribotype IV, 12 strains) belonged to group D. Moreover, 11 strains and 1 strain, respectively, belonged to the nonpathogenic groups A and B1. This is in keeping with our previous data on extraintestinal *E. coli*: 68% of neonatal *E. coli* meningitis isolates belonged to phylogenetic group B2, and 22, 6, and 4% belonged to groups D, A, and B1, respectively (5). Very recently, Johnson et al. (26), studying *E. coli* bacteremia strains from diverse sources, found a prevalence of groups A, B2, and D similar to that observed here. However, those authors found a significantly higher proportion of B1 strains (19 of 189 versus 1 of 100; $P = 0.006$), possibly because of the heterogeneity of the primary sites of infection from which their strains were derived. It has been reported that most *E. coli* blood isolates from immunocompromised hosts exhibit the B1 carboxylesterase phenotype, defined as belonging to groups other than B2, and are devoid of virulence factors (25, 27, 40). Overall, our results agree with these latter reports, as group A and B1 strains were more frequent in compromised patients than in noncompromised patients (15.2 versus 7.3%).

Few authors have studied the prevalence of virulence genes in community-acquired urosepsis *E. coli* isolates (29, 31). The proportions of isolates harboring *papC*, *cnf-1*, *hly*, and *aer* in our study (78, 21, 34, and 80%, respectively) are comparable to those found in blood isolates by Johnson and Stell (77, 16, 41, and 80%, respectively) (31). The prevalence of these genes was different in bacteremia isolates studied by Hilali et al. (20) and Maslow et al. (35), but this may be explained by the diversity of the primary source of infection in the latter studies.

Geographic location may also influence the distribution of *papG* alleles in *E. coli* strains causing bacteremia (22, 24, 37). Allele II was the predominant *papG* allele among *E. coli* blood isolates from adults in Seattle, Wash. (22); Long Beach, Calif. (24); Nairobi, Kenya (24); and Sweden (22, 37). In contrast, *papG* allele III was found by Johnson et al. in a majority of isolates from Boston, Mass. (24). Allele II predominated (68%) over allele III in our *E. coli* strains isolated from French adults with bacteremia and urinary tract infection. Moreover, the *papG* allele distribution differed according to host compromise status. The observed associations between *papGII* and noncompromised hosts and between *papG* negativity and host compromise in our study are consistent with previous reports (22, 37). Host compromise factors facilitate bloodstream invasion by *papG*-negative *E. coli* strains (21). Five percent of our strains were positive for *papC* and negative for *papG* alleles I, II, and III, in keeping with previous reports (26, 34, 37). This suggests that another allele may be present or that *papG* may be deleted. Interestingly, four of these five strains belonged to phylogenetic group A.

Extraintestinal pathogenic *E. coli* isolates are known to carry large chromosomal regions required for virulence; these so-called PAIs have been defined in uropathogenic strains 536, J96, and CFT073. We found various combinations of the genes studied here. The concomitant presence of *fyuA*/*irp-2* (encoding an iron uptake system representative of the *Yersinia* high-pathogenicity island [HPI]) (1) was found in 92% of our strains and thus seems to be characteristic of *E. coli* blood isolates. A

similar high level has previously been found in *E. coli* urosepsis isolates (31, 43). *fyuA/irp-2* was found in all of our isolates belonging to phylogenetic group A. Similarly, these genes have been found in all *E. coli* neonatal meningitis strains belonging to group A (10). In contrast, *fyuA/irp-2* was found in only 32% of ECOR group A strains (10) and in 30% of fecal isolates (44). As phylogenetic group A strains are principal members of the commensal flora (12), our results suggest that commensal intestinal *E. coli* carrying *fyuA/irp-2* would have a selective advantage for causing bacteremia, although we did not perform functionality experiments. Johnson and Stell have suggested that HPI may constitute a useful target for preventive intervention (31). Likewise, from a practical point of view, the absence of *fyuA/irp-2* in a urinary tract isolate would tend to exclude the risk of bacteremia in noncompromised patients. Finally, the association of *papC* with *fyuA/irp-2* could be the minimal prerequisite for bacterial passage from a renal focus of infection into the bloodstream of noncompromised patients.

The association of *papGIII*, *hly*, *hra*, and *cnf-1* was observed in 14% of our strains, and these genes were located on a single DNA macrorestriction fragment. A similar prevalence of the physically linked combination of *papC*, *hly*, and *cnf-1* (19%) in *E. coli* isolates from cancer patients with septicemia has been reported (20). However, the authors of that study did not test for *papG* alleles. All of our strains bearing this combination belonged to group B2. The association of these four virulence genes, reported to be PAI II_{J96}-like genetic elements as in reference strain J96 (6), suggests the presence of a PAI II_{J96}-like domain in 14 of our 100 strains. In one of these strains (P89), the spontaneous en bloc deletion of these four genes provides strong evidence for the presence of such a PAI. Moreover, we found that the insertion site of the PAI II_{J96}-like domain was within *pheR* tRNA (as in reference strain J96) in 11 strains (including P89 and P100) and near *leuX* tRNA in four strains (including P100, which carried two copies of these four genes). Interestingly, we found that seven strains harbored *hly*, *hra*, and *cnf-1*, colocalized without *papGIII* and inserted within *pheR* tRNA. Among these seven strains, four carrying the *hly*, *cnf-1*, and *hra* association harbored *papGII* instead of *papGIII* on the same fragment. One of these four strains was genetically related to J96. This may point to a PAI II_{J96}-like domain, with allelic exchange of *papG* or the presence of *papG* allele II at another site and deletion of *papGIII* (31). In favor of the first possibility is the presence of *papGII*, *hly*, *cnf-1*, and *hra* on a 280-kb fragment in strain P52 (data not shown). The second possibility is supported by the fact that three of our strains carried the combination of *hly*, *cnf-1*, and *hra* on the same restriction fragment, without *pap*. Spontaneous deletion of *pap* has been documented (17). A similar combination of *cnf-1* and *hly* without *pap* was described by Hilali et al. for 4% of their *E. coli* blood isolates (20). It has been suggested that *E. coli* PAIs undergo additional recombination processes that lead to additions or deletions of genes within the PAI (18, 26, 45). These results, combined with the differences in PAI sizes and site-specific insertion that we found, point to genetic plasticity of PAI II_{J96}-like domains. However, the consistent physical linkage of *cnf-1*, *hly*, and *hra* that we found in 21 strains and the statistical association between *cnf-1* and *hly* found by other authors suggest that this cluster of three genes may constitute the backbone of the PAI and serve as specific markers of the PAI II_{J96}-like domain. PAI II_{J96}-like domains were found exclusively in group B2. This contrasts with the distribution of HPI in groups B2, D, and A and suggests that certain PAIs may be specifically restricted to particular phylogenetic groups. Moreover, our results suggest that the PAI II_{J96}-like domain is not restricted to a single clone, as we found it in four different ribotypes, in contrast to other reports (28, 30) (Table 4). Only two strains were genetically related to J96 by ribotyping, implying that archetypal strain J96 is a rare cause of urosepsis in France.

Genetic linkage of *papGII* to the *hly* locus, suggestive of the presence of a PAI I_{CFT073}-like domain (16, 32), was found in 16% of our isolates, a prevalence lower than that previously reported (16). Three of these strains also harbored a PAI II_{J96}-like domain (Table 2). Fifteen of these 16 strains also harbored the *aer* locus colocalized with *papGII* and *hly*. The smallest *Not*I DNA fragment containing the three genes was found in two strains and was approximately 200 kb long. Interestingly, in two strains (P9 and P89), *papGII* and *aer* were physically linked (without *hly*) on a *Not*I DNA fragment of 210 kb. This, combined with the fact that 93% of strains harboring *papGII* but not *hly* were *aer* positive, whereas *aer* is found at a significantly lower prevalence (53%) in *papGII*-negative strains, strongly suggests the presence of either a new PAI containing *papGII* and *aer* or a PAI I_{CFT073}-like domain from which *hly* has been deleted. However this needs to be supported by further experiments demonstrating the en bloc deletion of the genes belonging to the putative PAI. Further studies are under way to confirm our hypothesis.

Finally, three of our strains carried *papGII* without *hly*, *aer*, or *cnf-1*, which is compatible with a PAI II_{CFT073}-like domain (41), and one strain harbored *hly* alone, which is compatible with a PAI I_{536}-like domain (7, 18).

The distribution of virulence factors in our *E. coli* ribotype I urosepsis isolates is interesting. Indeed, the combination of *papGII* and *papGIII* was confined to ribotype I, while *papGIII* alone was present in isolates belonging to three other ribotypes. Previous studies have shown an association between serogroup 02 and *papGII* plus *papGIII* status (22, 24). The fact that *papGII* alone was present in ribotype I, in contrast to *papGIII*, suggests that *papGII* was acquired first by ribotype I strains. Thus, the combination of *papGIII*, *hly*, *cnf-1*, and *hra* (PAI II_{J96}-like domain) may have been acquired secondarily on a *papGII*-positive ribotype I background.

In conclusion, we characterized a collection of *E. coli* urosepsis isolates from French adults with respect to their phylogenetic background and virulence factor profile, in comparison with archetypal uropathogenic isolates. We found that most of the isolates belonged to phylogenetic groups B2 and D and harbored *papG* allele II and the *aer* and *fyuA/irp-2* genes. This study provides data on prevalence and phylogenetic distribution indicating direct genetic linkage between *pap* and certain virulence-associated genes, consistent with colocalization on PAIs, that have previously been shown to exhibit statistical associations in other *E. coli* strain collections. Moreover, we show the variability of such gene associations, suggesting the plasticity of archetypal PAIs, even if some gene associations may form the backbone of certain PAIs, such as the *hly*, *cnf-1*, and *hra* association in the PAI II_{J96}-like domain. PAI II_{J96}-like and PAI

I_{CFT073}-like domains were found in 21 and 16% of our strains, respectively, and three strains harbored both. Only one strain harbored a PAI I_{536}-like domain, and none harbored a PAI I_{J96}-like or PAI II_{536}-like domain, suggesting that these PAIs may not be important in the urosepsis mechanism.

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REFERENCES

1. **Bach, S., A. de Almeida, and E. Carniel.** 2000. The *Yersinia* high-pathogenicity island is present in different members of the family Enterobacteriaceae. FEMS Microbiol. Lett. **183:**289–294.
2. **Bingen, E., E. Denamur, N. Lambert-Zechovsky, C. Boissinot, N. Brahimi, Y. Aujard, P. Blot, and J. Elion.** 1992. Analysis of DNA restriction-fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. J. Infect. Dis. **165:**569–573.
3. **Bingen, E., E. Denamur, N. Lambert-Zechovsky, A. Bourdois, P. Mariani-Kurkdjian, J. P. Cezard, J. Navarro, and J. Elion.** 1991. DNA restriction-fragment length polymorphism differentiates crossed from independent infections in nosocomial *Xanthomonas maltophilia* bacteremia. J. Clin. Microbiol. **29:**1348–1350.
4. **Bingen, E., E. Denamur, B. Picard, P. Goullet, N. Lambert-Zechovsky, N. Brahimi, J. C. Mercier, F. Beaufils, and J. Elion.** 1992. Molecular epidemiology unravels the complexity of neonatal *Escherichia coli* acquisition in twins. J. Clin. Microbiol. **30:**1896–1898.
5. **Bingen, E., B. Picard, N. Brahimi, S. Mathy, P. Desjardins, J. Elion, and E. Denamur.** 1998. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J. Infect. Dis. **177:**642–650.
6. **Blum, G., V. Falbo, A. Caprioli, and J. Hacker.** 1995. Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae, and α-hemolysin form of the pathogenicity island II of the uropathogenic *Escherichia coli* strain J96. FEMS Microbiol. Lett. **126:**189–196.
7. **Blum, G., M. Ott, A. Lischwski, A. Ritter, H. Imrich, H. Tschäpe, and J. Hacker.** 1994. Excision of large DNA regions termed pathogenicity islands from tRNA-specific loci in the chromosome of an *Escherichia coli* wild-type pathogen. Infect. Immun. **62:**606–614.
8. **Boyd, E. F., and D. L. Hartl.** 1998. Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. J. Bacteriol. **180:**1159–1165.
9. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
10. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2001. The *Yersinia* high-pathogenicity island is highly predominant in virulence-associated phylogenetic groups of *Escherichia coli*. FEMS. Microbiol. Lett. **196:**153–157.
11. **Cross, A., I. Orskov, F. Orskov, J. Sadoff, and P. Gemski.** 1984. Identification of K1 *Escherichia coli* antigen. J. Clin. Microbiol. **20:**302–304.
12. **Duriez, P., O. Clermont, S. Bonacorsi, E. Bingen, A. Chaventre, J. Elion, B. Picard, and E. Denamur.** 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology **6:**1671–1676.
13. **Eisenstein, B. I., and G. W. Joes.** 1998. The spectrum of infections and pathogenic mechanisms of *Escherichia coli.* Adv. Intern. Med. **33:**231–252.
14. **Finlay, B. B., and S. Falkow.** 1997. Common themes in microbial pathogenicity revisited. Microbiol. Mol. Biol. Rev. **61:**136–169.
15. **Grothues, D., and B. Tümmler.** 1991. New approaches in genome analysis by pulsed-field gel electrophoresis: application to the analysis of *Pseudomonas* species. Mol. Microbiol. **5:**2763–2776.
16. **Guyer, D. M., J. S. Kao, and H. L. T. Mobley.** 1998. Genomic analysis of a pathogenicity island in uropathogenic *Escherichia coli* CFT073: distribution of homologous sequences among isolates from patients with pyelonephritis, cystitis, and catheter-associated bacteriuria and from fecal samples. Infect. Immun. **66:**4411–4417.
17. **Hacker, J., L. Bender, M. Ott, J. Wingender, B. Lund, R. Marre, and W. Goebel.** 1990. Deletions of chromosomal regions coding for fimbriae and hemolysins occur in vitro and in vivo in various extraintestinal *Escherichia coli* isolates. Microb. Pathog. **8:**213–225.
18. **Hacker, J., G. Blum-Oehler, B. Janke, G. Nagy, and W. Goebel.** 1999. Pathogenicity islands of extraintestinal *Escherichia coli*, p. 59–76. *In* J. B. Kaper and J. Hacker (ed.), Pathogenicity islands and other mobile virulence elements. American Society for Microbiology, Washington, D.C.
19. **Hacker, J., G. Blum-Oehler, I. Muhldorfer, and H. Tschape.** 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23:**1089–1097.
20. **Hilali, F., R. Ruimy, P. Saulnier, C. Barnabé, C. Le Bouguenec, M. Tibayrenc, and A. Andremont.** 2000. Prevalence of virulence genes and clonality in *Escherichia coli* strains that cause bacteremia in patients. Infect. Immun. **68:**3983–3989.
21. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
22. **Johnson, J. R.** 1998. *papG* alleles among *Escherichia coli* strains causing urosepsis: associations with other bacterial characteristics and host compromise. Infect. Immun. **66:**4568–4571.
23. **Johnson, J. R., and J. J. Brown.** 1996. A novel multiply-primed polymerase chain reaction assay for identification of variant *papG* genes encoding the Gal (α1–4)Gal-binding PapG adhesins of *Escherichia coli*. J. Infect. Dis. **173:**920–926.
24. **Johnson, J. R., J. J. Brown, and J. N. Maslow.** 1998. Clonal distribution of the three alleles of the Gal (α1–4) Gal-specific adhesin gene *papG* among *Escherichia coli* strains from patients with bacteremia. J. Infect. Dis. **177:**651–661.
25. **Johnson, J. R., P. Goullet, B. Picard, S. L. Moseley, P. L. Roberts, and W. E. Stamm.** 1991. Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. Infect. Immun. **59:**2311–2315.
26. **Johnson, J. R., T. T. O'Bryan, M. Kuskowski, and J. N. Maslow.** 2001. Ongoing horizontal and vertical transmission of virulence genes and *papA* alleles among *Escherichia coli* blood isolates from patients with diverse-source bacteremia. Infect. Immun. **69:**5363–5374.
27. **Johnson, J. R., I. Orskov, F. Orskov, P. Goullet, B. Picard, S. L. Moseley, P. L. Roberts, and W. E. Stamm.** 1994. O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing urosepsis. J. Infect. Dis. **169:**119–126.
28. **Johnson, J. R., T. A. Russo, F. Scheutz, J. J. Brown, L. Zhang, K. Palin, C. Rode, C. Bloch, C. F. Marrs, and B. Foxman.** 1997. Discovery of disseminated J96-like strains of uropathogenic *Escherichia coli* O4:H5 containing genes for both PapG (J96) (class I) and PrsG (J96) (class III) Gal(α1–4) Gal-binding adhesins. J. Infect. Dis. **175:**983–988.
29. **Johnson, J. R., T. A. Russo, P. I. Tarr, U. Carlino, S. S. Bilge, J. C. Vary, Jr., and A. L. Stell.** 2000. Molecular epidemiological and phylogenetic associations of two novel putative virulence genes, *iha* and *iroN* (*E. coli*), among *Escherichia coli* isolates from patients with urosepsis. Infect. Immun. **68:**3040–3047.
30. **Johnson, J. R., A. E. Stapleton, T. A. Russo, F. Scheutz, J. J. Brown, and J. N. Maslow.** 1997. Characteristics and prevalence within serogroup O4 of a J96-like clonal group of uropathogenic *Escherichia coli* O4:H5 containing the class I and class III alleles of *papG*. Infect. Immun. **65:**2153–2159.
31. **Johnson, J. R., and A. L. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
32. **Kao, J. S., D. M. Stucker, J. W. Warren, and H. L. T. Mobley.** 1997. Pathogenicity island sequences of pyelonephritogenic *Escherichia coli* CFT073 are associated with virulent uropathogenic strains. Infect. Immun. **65:**2812–2820.
33. **Le Bouguenec, C., M. Archambaud, and A. Labigne.** 1992. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J. Clin. Microbiol. **30:**1189–1193.
34. **Marklund, B. I., J. M. Tennent, E. Garcia, A. Hamers, M. Baga, F. Lindberg, W. Gaastra, and S. Normark.** 1992. Horizontal gene transfer of the *Escherichia coli pap* and *prs* pili operons as a mechanism for the development of tissue-specific adhesive properties. Mol. Microbiol. **6:**2225–2242.
35. **Maslow, J. N., T. S. Whittam, C. F. Gilks, R. A. Wilson, M. E. Mulligan, K. S. Adams, and R. D. Arbeit.** 1995. Clonal relationships among bloodstream isolates of *Escherichia coli*. Infect. Immun. **63:**2409–2417.
36. **Ott, M., L. Bender, G. Blum, M. Schmittroth, M. Achtman, H. Tschäpe, and J. Hacker.** 1991. Virulence patterns and long-range genetic mapping of extraintestinal *Escherichia coli* K1, K5, and K100 isolates: use of pulsed-field gel electrophoresis. Infect. Immun. **59:**2664–2672.
37. **Otto, G., M. Magnusson, M. Svensson, J. H. Braconnier, and C. Svanborg.** 2001. *pap* genotype and P fimbrial expression in *Escherichia coli* causing bacteremic and nonbacteremic febrile urinary tract infection. Clin. Infect. Dis. **32:**1523–1531.
38. **Picard, B., P. Duriez, S. Gouriou, I. Matic, E. Denamur, and F. Taddei.** 2001. Mutator natural *Escherichia coli* isolates have an unusual virulence phenotype. Infect. Immun. **9:**9–14.
39. **Picard, B., J. S. Garcia, S. Gouriou, P. Duriez, N. Brahimi, E. Bingen, J. Elion, and E. Denamur.** 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect. Immun. **67:**546–553.
40. **Picard, B., and P. Goullet.** 1988. Correlation between electrophoretic types

B1 and B2 of carboxylesterase B and host-dependent factors in *Escherichia coli* septicaemia. Epidemiol. Infect. **100:**51–61.
41. **Rasko, D. A., J. A. Phillips, X. Li, and H. L. T. Mobley.** 2001. Identification of DNA sequences from a second pathogenicity island of uropathogenic *Escherichia coli* CFT073: probes specific for uropathogenic populations. J. Infect. Dis. **184:**1041–1049.
42. **Russo, T. A., and J. R. Johnson.** 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J. Infect. Dis. **181:**1753–1754.
43. **Schubert, S., S. Cuenca, D. Fischer, and J. Heesemann.** 2000. High-pathogenicity island of *Yersinia pestis* in *Enterobacteriaceae* isolated from blood cultures and urine samples: prevalence and functional expression. J. Infect. Dis. **182:**1268–1271.
44. **Schubert, S., A. Rakin, H. Karch, E. Carniel, and J. Heesemann.** 1998. Prevalence of the "high-pathogenicity island" of *Yersinia* species among *Escherichia coli* strains that are pathogenic to humans. Infect. Immun. **66:** 480–485.
45. **Swenson, D. L., N. O. Bukanov, D. E. Berg, and R. A. Welch.** 1996. Two pathogenicity islands in uropathogenic *Escherichia coli* J96: cosmid cloning and sample sequencing. Infect. Immun. **64:**3736–3743.

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