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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*

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The recent discovery of a geographically dispersed clonal group of *Escherichia coli* O4:H5 that includes prototypic uropathogenic strain J96 prompted us to determine the prevalence of J96-like strains within serogroup O4 and to further assess the characteristics of such strains. We used O:K:H;F serotyping, PCR-based genomic fingerprinting, pulsed-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MLEE), and PCR detection of the three *papG* alleles and of the cytotoxic necrotizing factor 1 (*cnf1*) and aerobactin (*aer*) gene sequences to characterize the 15 O4 strains among 336 *E. coli* isolates from three clinical collections (187 from mixed-source bacteremia, 75 from urosepsis, and 74 from acute cystitis). J96-like strains constituted approximately half of the O4 strains, or 2% of the total population. In contrast to other O4 strains, the J96-like strains characteristically exhibited specific group III capsular antigens, the H5 flagellar and F13 fimbrial antigens, a distinctive PCR genomic fingerprint, the class III *papG* allele (plus, in 50% of strains, the enigmatic class I *papG* allele), and *cnf1* but lacked *aer*. A subset of these strains was remarkably homogeneous with respect to all these characteristics and exhibited a distinctive PFGE fingerprint and MLEE pattern. These findings clarify the epidemiological relevance of J96 as a model extraintestinal pathogen, provide further evidence of the class I *papG* allele outside of strain J96, and offer insights into the evolution of *E. coli* serogroup O4.

Pathogenic strains of *Escherichia coli* that cause urinary tract infection, bacteremia, meningitis, and other extraintestinal infections typically belong to certain genetic lineages, or virulent clonal groups, that constitute a narrow subset of the total *E. coli* population (37, 39, 43, 58, 64). These virulent clonal groups are characterized by the expression of specific surface O, K, and H antigens as well as a variety of other properties that assist the organisms in overcoming host defenses and causing disease (7, 18, 41). They are globally distributed and collectively account for the majority of significant extraintestinal *E. coli* infections worldwide (7, 39). Improved understanding of the pathogenic clonal groups of *E. coli* and their virulence mechanisms and host associations can be expected to advance the development of preventive and therapeutic measures for the costly (45), morbid, and sometimes fatal infections that these strains cause.

Virulence properties that are of suspected or documented importance in the pathogenesis of extraintestinal *E. coli* infections include P fimbrial adhesins, the toxins alpha-hemolysin and cytotoxic necrotizing factor, the aerobactin iron sequestration system, *guaA* and *argC*, group II and III polysaccharide capsules, and lipopolysaccharide (4, 6, 7, 18, 41, 46, 51, 52). Of these, the evidence for a central role in uropathogenesis is strongest for P fimbriae (7, 18). P fimbriae mediate attachment to host intestinal, vaginal, and urinary epithelial surfaces via lectin-like binding to Gal(α1-4)Gal-containing isoreceptors on host tissues (7, 18, 60). This binding is mediated by PapG (pyelonephritis-associated pilus) adhesin molecules (12, 33) located at the fimbrial tips on flexible fibrillae (29). PapG occurs in three known molecular variants (classes I to III), each of which is encoded by a corresponding *papG* allele: *papG* from J96 (class I), *papG* from IA2 (and other strains) (class II), and *pap-2G* or *prsG* from J96 (class III) (35). The three PapG variants exhibit subtly different preferences for substituents on the consensus Gal(α1-4)Gal core receptor which may be important in determining the pathogen's host range or tissue tropism (16, 26, 30, 31, 62).

Pyelonephritis isolate J96 (13), previously reported to be of serotype O4:K6:H5 (32), has served since the late 1970s as a model pathogen for the investigation of the pathogenesis of extraintestinal *E. coli* infections. It was the source for the first cloned *pap*, *pap-2* (*prs*), hemolysin, and type 1 fimbrial operons (13, 25, 34, 66) and has been used extensively in animal models of urinary tract infection and for in vitro adherence assays (11, 13–16, 28, 47, 56, 62). Recognition of the three variants of *papG* in the late 1980s (25, 32, 34) and the creation of DNA probes specific for each *papG* variant for use in molecular epidemiological surveys (35) led to the perplexing discovery that the initial *papG* variant from strain J96 could not be found outside of strain J96 (17). This finding suggested that the class I *papG* allele might be unique to strain J96 (17), which called into question the relevance of the class I PapG adhesin to pathogenesis and also the relevance of J96 as a model extraintestinal pathogen.

Johnson et al. recently described a disseminated clonal group of virulent *E. coli* O4:H5 that includes strain J96 and is

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TABLE 1. Primers used in study

Target DNA	Primer	Orientation	Coordinates	Sequence (5′-3′)	Reference
Aerobactin (*iutA*)	aer-851f	Forward	851–872	ggctggacatcatgggaactgg	This study
	aer-1152r	Reverse	1152–1132	cgtcgggaacgggtagaatcg	This study
Cytotoxic necrotizing factor 1 (*cnf1*)	cnf-1833f	Forward	1833–1856	cagtgaagctcaacgagactatgc	10
	cnf-2965r	Reverse	2965–2944	caggaggcgttgatggctcagg	10
papG (class I)	j96f-193	Forward	193–213	tcgtgctcaggtccggaattt	19
	j96r-653	Reverse	653–633	tggcatcccccaacattatcg	19
papG (class II)	ia2-383f	Forward	383–402	gggatgagcgggcctttgat	19
	ia2-572r	Reverse	572–554	cgggcccccaagtaactcg	19
papG (class III)	prs-198f	Forward	198–219	ggcctgcaatggatttacctgg	19
	prs-455r	Reverse	455–434	ccaccaaatgaccatgccagac	19
ERIC sequences	ERIC 1R	NA[a]	NA	atgtaagctcctggggattcac	65
	ERIC 2	NA	NA	aagtaagtgactggggtgagcg	65
BOX sequences	BOX A1R	NA	NA	ctacggcaaggcgacgctgacg	65

[a] NA, not applicable.

characterized by the presence of both the class I and class III alleles of *papG* (23). These findings indicated that the class I *papG* variant is not unique to strain J96 and suggested that J96-like strains may be more prevalent among clinical isolates than previously thought (17, 44, 48). However, the small sample size and the selection factors used in this initial study (23) precluded meaningful epidemiological conclusions regarding the prevalence of J96-like strains among clinical isolates.

Therefore, we undertook this study to define the prevalence and other characteristics of the J96-like clonal group and its class I *papG* allele within serogroup O4 through the examination of a nonpreselected group of strains of *E. coli* O4 from three different serotyped collections of pathogenic *E. coli*, each representing a different geographic locale and/or clinical infection syndrome (24). For the present study, these strains were typed with respect to O, K, H, and F antigens, underlying genomic background, and specific virulence factor (VF) genes, including the three *papG* alleles and the genes encoding cytotoxic necrotizing factor 1 (*cnf1*) and aerobactin (*aer*). The results reveal that the J96-like clonal group is widely dispersed across North America and that it constitutes approximately half of all O4 strains causing bacteremia, urosepsis, or acute cystitis in the United States.

(This work has been presented in part elsewhere [24].)

MATERIALS AND METHODS

Source of strains. All 15 *E. coli* strains of serogroup O4 from three clinical collections were examined. The first collection comprised 187 blood culture isolates from patients with bacteremia from diverse sources, collected in Boston, Mass., Long Beach, Calif., and Nairobi, Kenya, from 1988 through 1991 (38). The virulence characteristics and clonal structure of these strains have been previously reported (38). The second collection comprised 75 blood culture isolates from patients with urosepsis, collected at four hospitals in Seattle, Wash., in the mid-1980s. The virulence characteristics and serotypes of these strains, and associations of these properties with host characteristics, have been previously reported (20–22). One of these strains (PM8) was included as a control in the initial description of the J96-like clone (23). The third collection comprised 74 urine isolates from young women with recurrent (n = 40) or first-episode (n = 34) acute cystitis, collected in Seattle in the late 1980s (60). The virulence characteristics of these strains also have been previously reported (60), as have the glycolipid binding characteristics of strain R45, an O4 isolate from this collection (61). Five O4:H5 strains that were shown in a previous study to be members of the J96-like clonal group, including CP9 (a bacteremia isolate from the National Institutes of Health, provided by T. A. Russo) (53, 54) and BF1023, BF1056, BF1040, and BF9043 (cystitis isolates from Austin, Tex., and Ann Arbor, Mich., provided by Betsy Foxman) (10), were compared with the 15 O4 strains from the above-mentioned clinical collections. Prototypic uropathogenic strains J96 (13) and IA2 (5), provided by Barbara Minshew (University of Washington, Seattle) and Steve Clegg (University of Iowa, Iowa City), respectively, were used as positive controls.

Serotyping. Initial O antigen typing was done at the *E. coli* Reference Center, Pennsylvania State University, University Park, for the bacteremia and cystitis collections and at the International *Escherichia* and *Klebsiella* Centre (World Health Organization), Copenhagen, Denmark, for the urosepsis collection. Strains identified as O4 underwent complete O:K:H serotyping and F fimbrial antigen typing by the International *Escherichia* and *Klebsiella* Centre. Lipopolysaccharide (O), capsular (K), and flagellar (H) antigens were determined by using the established typing sera and methods as specified by Orskov and Orskov (40). Fimbrial (F) antigen determination was done by using rocket immunoelectrophoresis followed by crossed-line immunoelectrophoresis as previously described (42).

PCR assays for *papG* alleles, *cnf1*, and *aer*. *papG* allele genotypes were determined using a multiply primed *papG* PCR assay (19), with modifications. Primers are listed in Table 1. Amplification was done by using all three primer pairs simultaneously in single-tube 50-μl reaction mixtures as previously described (19), except that each primer's concentration was 0.45 μM, and the thermal cycler protocol was as follows: denaturation at 95°C for 7 min; then 10 cycles of denaturation (94°C, 1 min), annealing (68°C, 2 min), and extension (72°C, 3 min); then 15 cycles of denaturation (94°C, 1 min) and annealing/extension (72°C, 4 min); and then a final extension (72°C, 10 min). Allele-specific *papG* PCR products were resolved in a single gel lane by agarose gel electrophoresis (19). Primers for *cnf1* (9) were as previously described (10), and those for *aer* were selected based on the published sequence of the aerobactin receptor gene *iutA* (28) (Table 1). Target DNA preparation and thermal cycler protocol for *cnf1* and *aer* were as for *papG* PCR. Results of all PCR-based virulence factor assays were confirmed with a replicate determination involving separately prepared target DNA. Additional assays were done as needed to resolve discrepancies when they occurred.

Rep-PCR and PFGE. Genomic fingerprints were generated using repetitive element PCR (Rep-PCR) with the enterobacterial repeat intergenic consensus sequence (ERIC) primers ERIC1R and ERIC2 and the BOX A1R primer as described by Versalovic et al. (65) (Table 1). Target DNA for Rep-PCR was prepared by the whole-cell boiled lysate method of Woods et al. (68). For pulsed-field gel electrophoresis (PFGE), whole chromosomal DNA in agarose was digested with *Xba*I, and the restriction fragments were separated in a CHEF DRII apparatus (Bio-Rad, Richmond, Calif.) with 0.5× Tris-borate-EDTA buffer at 200 V and 15°C for 22 h and pulse times from 1 to 40 s with linear ramping, as previously described (36).

MLEE. Multilocus enzyme electrophoresis (MLEE) was done by Thomas Whittam, using 20 metabolic enzymes as previously described (57, 67). Electromorphs of each enzyme, determined by comparison with standard mobility variants, were equated with alleles at the corresponding chromosomal gene locus. Distinctive multilocus genotypes (allele combinations) were designated electrophoretic types (ETs), which were numbered as previously described (37).

Statistical methods. Comparisons of proportions were tested by using Fisher's exact test.

RESULTS

Serotypes of J96-like strains and other strains. The 15 O4 strains, which constituted 4.5% of the 336 strains in the three clinical collections, were first sorted according to O:K:H;F serotypes, since in the initial report of the J96-like clonal group, specific K, H, and F antigens were identified as characteristic of J96-like strains (23). In the present study, strains segregated cleanly according to serotype into two equal size groups. Seven (47%) of the O4 strains exhibited only previously defined J96-like antigens (23), i.e., distinctive group III

TABLE 2. Characteristics of J96-like and other strains of *E. coli* O4

Group	Strain[a]	Syndrome	Location	K:H;F serotype[b]			Genotype[c]		ET	Other VF gene[d]		*papG* allele		
				K	H	F	PFGE	BOX		*cnf1*	*aer*	I	II	III
Control	J96	Pyelonephritis	Seattle	K−	H5	13	A	A	73	+	−	+	−	+
J96-like strains	CA 002	Urosepsis	Long Beach	K3	H5	13	A	A	73	+	−	−	+	+
	CA 022	Urosepsis	Long Beach	K3	H5	13	B	A	73	+	−	−	+	−
	CA 062	Urosepsis	Long Beach	K3	H5	13	C	A	20	+	−	−	−	+
	R28	Cystitis	Seattle	K3	H5	13	D	A	73	+	−	+	−	+
	BOS 038	Sepsis, pneumonia	Boston	K10,K54/96	H−	13	E	A	20	+	−	+	−	+
	BOS 110	Sepsis, unknown source	Boston	K10,K54/96	H5	13	F	A	20	+	−	+	−	+
	518	Cystitis	Seattle	K10,K54/96	H5	13	G (F)	A	20	+	−	+	−	+
Other O4 strains	AFR 015	Urosepsis	Nairobi	K+	H1	16, 11	H	B	73	+	+	−	+	−
	BOS 021	Urosepsis	Boston	K+	H−	16	I	B	73	−	+	−	+	−
	BOS 040	Urosepsis	Boston	K+	H1	16, 11	J	B	73	+	+	−	+	−
	BOS 046	Urosepsis	Boston	K12	H−	16	K	B	73	−	+	−	+	−
	BOS 105	Urosepsis	Boston	K7	H−	16	L	A	73	+	−	−	−	+
	R45	Cystitis	Seattle	K12	H1	16	M (L)	B	73	+	+	−	+	−
	V31	Urosepsis	Seattle	K12	H1	11	N	B	73[e]	+	−	−	+	−
	PM8	Urosepsis	Seattle	K12	H−	16	O	B	73	−	+	−	+	−

[a] BOS 038 was from a neutropenic patient with pneumonia; BOS 110 was from a patient with bacteremia of unknown source.
[b] K10-positive strains also reacted with both K96 and K54 (which cross-react). K+, capsule positive but not K typeable.
[c] Patterns with the same letter are similar or indistinguishable if in the same column and are unrelated if in different columns. Letters in parentheses indicate close similarity to the pattern of the strain in same column with same letter.
[d] *cnf1*, cytotoxic necrotizing factor 1; *aer*, aerobactin (*iutA*).
[e] Partial ET determination only (similar to ET 73 with respect to key enzymes malate dehydrogenase and β-galactosidase).

capsules (specifically K3 or K10,K54/K96), the H5 flagellar antigen, and the F13 fimbrial antigen, and so were operationally defined as J96-like (Table 2). The other eight strains had only non-J96-like antigens, i.e., K7, K12, or untypeable capsule, the H1 flagellar antigen (or were nonmotile), and the F16 and/or the F11 fimbrial antigen, and so were operationally defined as "other" (Table 2).

***papG* alleles.** The 15 O4 strains all exhibited one or more of the three *papG* alleles, in patterns that corresponded closely with the serotype-based stratification into J96-like versus other O4 strain groups (Fig. 1; Table 2). Of the seven serologically J96-like strains, all but one (CA 022) had the class III *papG* allele, which occurred four times in combination with the class I allele (as in strain J96), once in combination with the class II allele, and once as the sole *papG* allele. The class I allele occurred only among the J96-like strains, always together with the class III allele. Among the J96-like strains, the class II-only *papG* genotype occurred only once, in strain CA 022.

Statistical testing confirmed that the class I and class III *papG* alleles were significantly associated with J96-like status, whereas the class II allele was significantly associated with non-J96-like status (Tables 2 and 3). Among the J96-like strains, the class I *papG* allele appeared to segregate according to geographic locale, being absent among the three Long Beach strains but present in the other four J96-like strains, which were from Boston and Seattle ($P = 0.03$, Long Beach versus others) (Table 2). Comparisons between the *papG* alleles among all 15 O4 strains revealed a significant positive association between the class I and class III alleles (12 of 15 strains concordantly positive or negative for classes I and III, compared with 3 of 15 discordant [$P = 0.03$]), in contrast to the highly significant negative associations observed between each of these alleles and the class II allele (versus class I, 2 of 15 concordant, compared with 13 of 15 discordant [$P = 0.01$]; versus class III, 1 of 15 concordant, compared with 14 discordant [$P = 0.001$]).

PFGE and Rep-PCR genomic fingerprints. Restriction fragment patterns generated by PFGE of *Xba*I-digested total DNA were unique to individual strains (Table 2), with notable exceptions in which similarities were observed between strains within either the J96-like or the other O4 strain group, but not between these two groups. First, within the J96-like group, strain CA 002 (O4:K3:H5;F13) was essentially indistinguishable by PFGE from strain J96 itself (O4:K−:H5;F13). CA 002 thus provided the closest match to J96 (by PFGE criteria) of any of the J96-like strains either in this study or in the initial report of the J96-like clonal group (23), despite CA 002's divergent *papG* allele configuration (II+III, compared with J96's I+III [Table 2]). Second, two other J96-like strains (BOS 110, a Boston bacteremia isolate, and 518, a Seattle cystitis isolate) had essentially indistinguishable PFGE patterns. These strains both expressed the K10 and K54/K96 capsular antigens and shared J96's I+III *papG* allele configuration and *cnf1*-positive, *aer*-negative status. Finally, two of the non-J96-like strains (Boston bacteremia isolate BOS 105 and Seattle cystitis isolate R45) also had essentially indistinguishable PFGE patterns. However, these two strains differed with respect to K antigen, BOX PCR pattern, *papG* allele configuration, and aerobactin status (Table 2).

Rep-PCR fingerprints generated by using the ERIC primers were fairly similar among the 15 O4 strains and did not clearly discriminate between J96-like and other strains (not shown). In contrast, although BOX PCR fingerprints demonstrated many common bands as well as some variable bands among the 15 strains (Fig. 1), J96 and the seven J96-like strains all exhibited a distinctive band at ca. 300 bp that was similar in appearance to that previously described for J96-like strains (23). In contrast, this band was absent from all of the other O4 strains except BOS 105 (Fig. 1).

MLEE results. The 15 O4 strains segregated into two closely related ETs, ET 20 and ET 73 (36), that differed at only one locus (malate dehydrogenase) (Table 2). Paradoxically, this ET split did not correspond with the division into J96-like and other strains as suggested by serotype, *papG* allele pattern, PFGE, and BOX PCR fingerprints (Table 2). Instead, approximately half of the J96-like strains were grouped into ET 20, which contained only J96-like strains, whereas the other J96-like strains (and J96 itself) were grouped with the non-J96-like

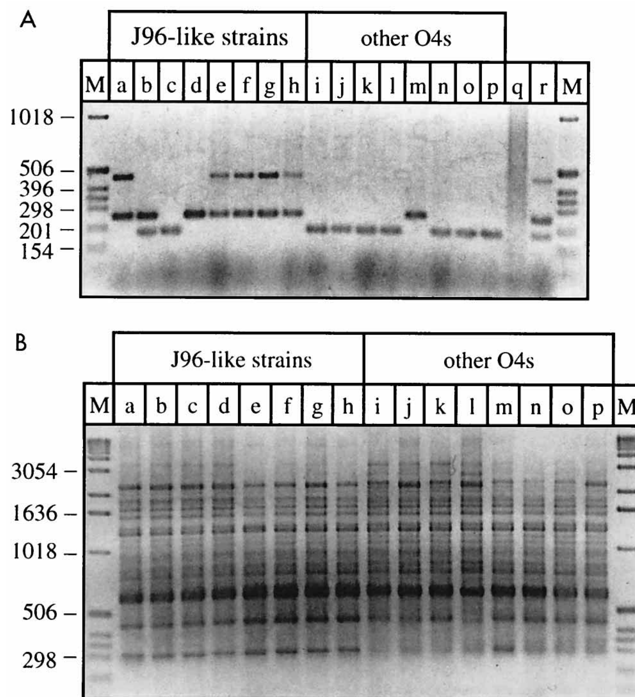

FIG. 1. Allele-specific *papG* PCR (A) and BOX PCR (B) of *E. coli* O4 strains. Lanes: M, molecular weight marker; a, J96; b, CA 002; c, CA 022; d, CA 062; e, R28; f, BOS 038; g, BOS 110; h, 518; i, AFR 015; j, BOS 021; k, BOS 040; l, BOS 046; m, BOS 105; n, R45; o, V31; p, PM8; q (A only), blank; r (A only), J96 plus IA2. (A) *papG* PCR. All but one of the J96-like strains exhibits the class III *papG* PCR product (middle band, 258 bp); four also have the class I *papG* PCR product (high band, 461 bp). All other O4 strains exhibit only the class II *papG* PCR product (low band, 190 bp). (B) BOX PCR. All J96-like strains exhibit a prominent ca. 300-bp band just above the 298-bp marker that is absent from all of the other O4 strains except BOS 105. (The 300-bp band was digitally enhanced for clarity.)

strains in the larger ET 73 (Table 2). There was good correspondence of ET with K antigens and *papG* alleles: the K10 and K54/K96 antigens were confined to ET 20, and with a single exception each, the K3 antigen was confined to ET 73 and the class I *papG* allele was confined to ET 20 (Table 2). Concordance between ET and PFGE pattern was seen with J96 and its PFGE look-alike, CA 002 (both ET 73), as well as with the paired J96-like strains BOS 110 and 518 (both ET 20) and the paired non-J96-like strains BOS 105 and R45 (both ET 73) (Table 2).

Other VF genes. By PCR, *cnf1* sequences were present in all of the J96-like strains but absent from three of the eight other O4 strains ($P = 0.20$) (Tables 2 and 3). In contrast, *aer* sequences were absent from all of the J96-like strains but present in 75% of the other O4 strains ($P = 0.007$) (Tables 2 and 3). The *cnf1*-positive, *aer*-negative pattern occurred in all the J96-like strains but in only two of the other O4 strains ($P = 0.007$), one of which was BOS 105 (Table 2).

Comparison to previously identified J96-like strains. Strains BOS 110 and 518, J96-like strains from the present study which were similar to one another by PFGE (Table 2), also were indistinguishable by PFGE from the previously described J96-like strains CP9, BF1023, and BF1056 (not shown). The latter strains had been found to have the same PFGE fingerprint and, like BOS 110 and 518, to express the O4, K10, K54/K96, H5, and F13 antigens and to exhibit J96's I+III *papG* allele configuration (23). MLEE was concordant with PFGE for both the old and new look-alike strains, showing them all to belong to the minority ET 20 (Table 2 and not shown). In contrast, the other two previously described J96-like cystitis isolates (23) were found to belong to ET 73 (BF1040) and a related ET that differed at only one locus (β-galactosidase) (BF9043). The latter two strains also had differing PFGE fingerprints that were similar to but distinct from those of J96, CP9, BF1040, and BF9043 (23) and from those of the J96-like strains in the present study (not shown).

Geographic and clinical associations. O4 strains were similarly distributed among the three collections studied, accounting for 10 (5%) of the 187 mixed-source bacteremia isolates, two (3%) of the 75 urosepsis isolates, and three (4%) of the 74 cystitis isolates. Although J96-like strains appeared to segregate according to collection (five [3%] of the mixed-source bacteremia isolates and two [3%] of the 74 cystitis isolates, but none of the urosepsis isolates, were J96-like), these differences were not statistically significant ($P > 0.10$, distribution of J96-like status according to source). All three O4 strains from Long Beach (CA 002, CA 022, and CA 062) were J96-like, compared with only four (33%) of the other 12 O4 strains ($P = 0.08$) (Table 2).

DISCUSSION

We describe in detail the genotypic and phenotypic characteristics of 15 *E. coli* isolates of serogroup O4 from among 336 extraintestinal infection isolates from three clinical collections. These strains were studied to determine the prevalence of a recently described J96-like clonal group (23) within *E. coli* O4 and to gain a better understanding of the characteristics of such strains, including the bacteriological and clinical correlates of their enigmatic class I *papG* allele (17).

We found that the J96-like clonal group, as defined initially by serological criteria and subsequently by multiple genotypic criteria, accounted for approximately half of the O4 strains, which in turn constituted 4.5% of the total study population. J96-like strains were found in Boston and Long Beach, which extends the documented geographic range of the J96-like clonal group beyond the previously identified sites of Seattle, Austin, Ann Arbor, and Bethesda, Md. (23). We found a similar prevalence of J96-like strains among O4 isolates from the east and west coasts of the United States and among cystitis compared with bacteremia isolates. Thus, our findings suggest that this clonal group accounts for at least 2% of extraintestinal pathogenic *E. coli* organisms causing bacteremia, urosepsis, and uncomplicated cystitis in adults across North America. Whether this clonal group is similarly prevalent among O4 strains from other clinical syndromes, hosts, and geographic locales, and whether it extends beyond serogroup O4, remains to be determined.

TABLE 3. Distribution of *papG* alleles, *cnf1*, and *aer* according to J96-like status

Virulence factor gene	No. of strains positive		P value,[a] J96-like vs others
	J96-like ($n = 7$)	Other O4 strains ($n = 8$)	
Class I *papG*	4	0	0.03
Class II *papG*	2	7	0.04
Class III *papG*	6	1	0.01
cnf1	7	5	0.20
aer	0	6	0.007

[a] By Fisher's exact test.

Consistent with the initial description of the J96-like clonal group (23), in the present study the J96-like strains were characterized by presence of the class III *papG* allele and absence of the class II *papG* allele (Tables 2 and 3). Similarly, the class I *papG* allele, which until recently had been considered unique to source strain J96 (17), was found in several J96-like strains, always together with the class III allele, but was absent from non-J96-like O4 strains (Tables 2 and 3; Fig. 1). However, in contrast to the uniform presence of J96's I+III *papG* allele configuration among the five initially reported J96-like strains (23), only half of the J96-like strains in the present study exhibited the I+III *papG* genotype (Table 2). This difference between the two studies is likely attributable to a combination of sample size and selection bias, since four of the five previously reported J96-like strains were selected for special study partly based on known *pap* region similarities to J96 (23), which might have skewed the sample toward greater similarity to strain J96, including its specific *papG* allele genotype.

We identified several phenotypic and genotypic attributes that were highly characteristic of the J96-like clonal group. As in the initial report of J96-like strains (23), the K:H;F antigen serotype cleanly differentiated between J96-like and other strains. In the present study we initially used serotype to categorize O4 strains as to their J96-like status, and this stratification was subsequently substantiated by the results of multiple other assays, including those for background genomic characteristics and for specific VF genes (Table 2).

Although no single K antigen was consistently present among all members of the clonal group, all J96-like strains expressed one or more group III capsular antigens (46). In this regard, J96's capsule-negative status (which we have confirmed by using several different stocks of J96 from laboratories around the United States) (50) is of interest. J96 has been reported to be K6 (32); however, the *Escherichia* and *Klebsiella* Reference Centre was unable to confirm this result either in the past (55) or in the present study (Table 2). J96's similarity by PFGE to O4:K3 strain CA 002 (Table 2) suggests that J96 may actually be an occult K3. Since K3 (but not K6) is recognized as a group III capsule (46), K3 would be more consistent with the observed association of the J96-like clonal group with group III capsules (Table 2 and reference 23). However, the example of look-alike strains BOS 105 (K7) and R45 (K12) demonstrates that similar PFGE patterns do not necessarily equate with identical capsular types, probably because exchange of antigen-specific capsule synthesis genes may occur via horizontal transfer within a stable genomic background (59). Thus, ironically, despite our growing understanding of the capsular repertoire of the J96-like clonal group, the K type of strain J96 itself remains in question.

Both the H5 and F13 antigens were confined to the J96-like clonal group (Table 2) and were the only flagellar or fimbrial antigens expressed by J96-like strains in this study and the previous study (23). This suggests the possibility that within extraintestinal *E. coli* O4, the H5 antigen may equate with J96-like status. If this is so, the well-known pyelonephritogenic clone O4:K12:H5 (37, 57) may be closely related to strain J96. Supporting this hypothesis, some strains of serotype O4:K12:H5 (or H−) exhibit the J96-associated F13 antigen (1, 42). The two such strains that we have tested to date, strains 20025 (O4:K12:H−;F1C:F13:F14:F16) and C134-73 (O4:K12:H5; F13:F16) (1, 42), both exhibit a class III-only *papG* genotype and have the J96-like ca. 300-bp band by BOX PCR (55), consistent with membership in the J96-like clonal group. Whether these strains also have other typical J96-like features, and whether J96-like characteristics are shared by other O4:K12:H5 strains, remains to be determined.

The distinctive ca. 300-bp DNA fragment that is consistently observed by BOX PCR among J96-like strains is of uncertain significance but presumably reflects an arrangement of BOX sequences on the genome of J96-like strains that is usually absent from other O4 strains (Table 2; Fig. 1). The presence of this band in strain BOS 105, together with this strain's class III-only *papG* configuration and its *cnf1*-positive, *aer*-negative status, places BOS 105 in a somewhat ambiguous intermediate position between the J96-like and other O4 strains (Table 2). This strain's similarity with respect to PFGE fingerprint (which, of the assays used in the present study, is the most definitive for identifying genetically similar strains) to the clearly non-J96-like strain R45, together with BOS 105's non-J96-like K, H, and F antigens (Table 2), led us to classify BOS 105 as non-J96-like for the present study. Examination of additional O4 strains should clarify the prevalence of such hybrids that span what otherwise appears to be a clean split within serogroup O4.

It was provocative to find all three *papG* alleles represented within the J96-like group, always in an F13 (PapA) background. Since the class I and class III *papG* alleles predominate among J96-like strains (Tables 2 and 3 and reference 23), the finding of an occasional class II allele (Table 2) suggests the occurrence of suboperonic recombinations between J96-like and non-J96-like *pap* operons, consistent with previous evidence of horizontal transfer of *pap* operons (or subcomponents thereof) between different *E. coli* lineages (2, 3, 35, 49). The patterns that we observed could have arisen either by horizontal transfer of an alien (i.e., class II) *papG* allele from a non-J96-like strain's non-F13 *pap* operon into a preexisting F13 *pap* operon in a J96-like strain or by acquisition of an entire alien *pap* operon by a J96-like strain, with subsequent in situ replacement of the alien (non-F13) *papA* allele with a J96-like F13 *papA* allele, possibly by internal recombination involving the J96-like strain's preexisting (F13+) *pap* operon (15). However, our data cannot exclude the possibility that the class II allele actually was the ancestral *papG* allele within the J96-like group. If so, some selection factor(s) must favor the class I and III alleles among J96-like strains, since these alleles have almost completely eclipsed the class II allele within this clonal group (Tables 2 and 3 and reference 23).

The universal presence of *cnf1* among the J96-like strains is not surprising, since in J96, *cnf1* is present on pathogenicity-associated island (PAI) V together with J96's class III *pap* operon and one of J96's two *hly* operons (8, 15, 37, 63). Our findings thus are consistent with the hypothesis that the J96-like strains all have the same PAI V as J96. If valid, this hypothesis (which remains to be experimentally confirmed) would suggest that (*cnf1*-positive) strain CA 022 either has a class II *papG* allele instead of class III allele in its PAI V *pap* operon or has lost the *pap* operon (or the *papG* portion thereof) from PAI V but has a class II *papG* allele in the *pap* operon of PAI IV, where J96 has a class I *papG* allele (37, 63).

In contrast to *cnf1*, the aerobactin system can be either chromosomal or plasmid borne (21), and when it is chromosomal, it is not known to be linked with other VF genes or to be part of a PAI. Thus, the fact that J96-like strains are uniformly *aer* negative, whereas other O4 strains are usually (but not always) *aer* positive (Table 2), is consistent with the concept that J96-like strains resemble one another (and are different from other O4 strains) in ways other than simply the possession of similar PAIs. In the present study, we did not test strains for VF genes which were previously reported to be uniformly present (*hly*, *sfa*, *ompT*, and *pil*) or absent (*drb*) among both J96-like and control strains (23), since we pre-

dicted these to be unlikely to differentiate between J96-like and other O4 strains.

It is noteworthy that although MLEE did identify two subsets within the J96-like group, it failed to detect the larger split within serogroup O4 between the J96-like and other O4 strains (Table 2). This perhaps is not altogether surprising, since whereas MLEE samples (nonselected) housekeeping genes, most of our other assays targeted virulence-associated properties that may be under selection pressure from the host and may be more genetically labile and mobile than the loci sampled by MLEE.

In summary, our data suggest that strains related to prototypic uropathogenic strain J96 constitute approximately 50% of *E. coli* serogroup O4 and approximately 2% of human extraintestinal *E. coli* isolates. The J96-like clonal group, which has been responsible for a variety of significant infections in adults across the United States, is characterized by specific group III capsules, the H5 flagellar and the F13 fimbrial antigens, the class III *papG* allele (plus, in 50% of strains, the enigmatic class I *papG* allele), a distinctive Rep-PCR genomic fingerprint with the BOX A1R primer, and *cnf1* positivity plus *aer* negativity. These findings demonstrate that the class I allele of *papG*, although present in a minority of O4 strains, is more prevalent than previously thought, which reopens the question of its contribution to urovirulence (14). These findings also confirm the epidemiological relevance of strain J96 as a model extraintestinal pathogen and provide insights into the evolution of *E. coli* serogroup O4.

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