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Aerobactin and Other Virulence Factor Genes among Strains of *Escherichia coli* Causing Urosepsis: Association with Patient Characteristics

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To assess the role of aerobactin as a virulence factor among uropathogenic *Escherichia coli*, we determined the prevalence, location, and phenotypic expression of aerobactin determinants among 58 *E. coli* strains causing bacteremic urinary tract infections. We correlated the presence of the aerobactin system with antimicrobial-agent resistance, the presence and phenotypic expression of other uropathogenic virulence factor determinants (P fimbriae, hemolysin, and type 1 fimbriae), and characteristics of patients. Colony and Southern hybridization of total and plasmid DNA with DNA probes for each virulence factor showed that aerobactin determinants were present in 78% of the strains and were plasmid associated in 21%, whereas P fimbria, hemolysin, and type 1 fimbria determinants were present in 74, 43, and 98% of the strains, respectively, and were always chromosomal. Chromosomal aerobactin, P fimbria, and hemolysin determinants occurred together on the chromosome more often in strains from patients without predisposing urological or medical conditions ($P = 0.04$). Strains with plasmid-encoded aerobactin lacked determinants for P fimbriae ($P = 0.004$) and hemolysin ($P = 0.0004$), were resistant to multiple antimicrobial agents ($P = 0.0001$), and were found only in compromised patients. Mating experiments demonstrated that some aerobactin plasmids also encoded antimicrobial-agent resistance. These findings suggest that the determinants for aerobactin, P fimbriae, and hemolysin are conserved on the chromosome of the antimicrobial-agent-susceptible uropathogenic strains of *E. coli* which invade noncompromised patients. In contrast, these chromosomal virulence factors are often absent from *E. coli* strains causing urosepsis in compromised hosts; these strains may acquire plasmid aerobactin in conjunction with antimicrobial-agent resistance genes.

Escherichia coli strains which cause urinary tract infections in patients without urological abnormalities typically possess specific virulence factors (such as hemolysin and P fimbriae) and belong to a limited number of O and K serogroups. These observations suggest the existence of uropathogenic clones of *E. coli* with enhanced virulence in the urinary tract (33). In contrast, in patients with predisposing medical or urological conditions, serious urinary tract infections are often caused by strains lacking these virulence factors (3, 15, 20).

Aerobactin, a bacterial siderophore, has recently been shown to be associated with *E. coli* strains which cause pyelonephritis and cystitis (5). To further assess the role of aerobactin as a virulence factor in uropathogenic *E. coli*, we undertook the present study to determine the prevalence, extent of expression, chromosomal-versus-plasmid location, and copy number of the genetic determinants for aerobactin, hemolysin, P fimbriae, and type 1 fimbriae among strains of *E. coli* from the blood of patients with urosepsis and to characterize interrelationships among these virulence factors. In addition, we sought to determine whether the presence and expression of aerobactin determinants are less common among *E. coli* strains from patients with urosepsis who have compromising urological or medical conditions. We also investigated the association between the presence of the determinants for these virulence factors and resistance to antimicrobial agents.

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MATERIALS AND METHODS

Patients and bacteria. Blood culture isolates of *E. coli* from 58 patients who had *E. coli* bacteremia arising from a urinary tract source were obtained from the clinical microbiology laboratories of four hospitals in Seattle, Wash. (Seattle Veterans Administration Medical Center, Harborview Medical Center, Pacific Medical Center, and University Hospital), as previously described (15). A single strain was obtained from each patient. Medical records were reviewed, as previously described, to determine whether compromising host conditions (including urinary tract abnormalities, urinary tract instrumentation, and serious medical illnesses) were present at the time of the bacteremic episode (15).

Preparation of probes. Probes used in the hybridization experiments were prepared from recombinant plasmids containing the sequences of interest. The appropriate DNA fragments, described below, were labeled with α-^{32}P-deoxyribonucleotides by nick translation as previously described (23). A 2-kilobase (kb) *Hin*dIII-*Pst*I fragment of pJHC-V12 (generously provided by M. Valvano and J. Crosa, Portland, Oreg.), which encodes a portion of a 63,000-dalton protein involved in aerobactin biosynthesis (6, 34), was used as a probe for the aerobactin operon. A 2-kb *Pst*I fragment of pWAM298 (generously provided by R. Welch, Madison, Wis.) (11), which is internal to the *hlyA* gene, was used as a probe for the hemolysin determinants. A 2.4-kb *Sma*I fragment of pRHU485, which encodes portions of the *papE*, *papF*, and *papG* genes, was used as a probe for the P fimbria operon (26, 31), and a 2-kb *Bam*HI-*Pst*I fragment of pSH2, which includes portions of the genes encoding an 86,000-

dalton protein and a 30,000-dalton protein required for type 1 fimbria expression (28), was used as a probe for the type 1 fimbria operon. (R. Hull and S. Hull, Houston, Tex., generously provided pRHU845 and pSH2.)

Isolation of total and plasmid DNA. Total DNA was extracted as previously described (4), and plasmid DNA was isolated by the procedure of Kado and Liu (16).

Southern blot analysis of total and plasmid DNA. Samples of total DNA from each strain were digested with four different restriction endonucleases or endonuclease combinations: *Bam*HI, *Hin*dIII-*Pst*I, *Sal*I, and *Eco*RI (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Unrestricted plasmid DNA and restricted total DNA were subjected to electrophoresis in 1.0% agarose and transferred to nylon filters (Nytran; Schleicher & Schuell, Inc., Keene, N.H.) after alkaline denaturation and neutralization as described by the manufacturer. Plasmid DNA gels were placed in 0.15 M HCl for 15 min before alkaline treatment.

Hybridization with DNA probes was performed at 37°C under high-stringency conditions (for nylon filters: 50% formamide–5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.5% sodium dodecyl sulfate [SDS]; rinse in 2× SSC–0.1% SDS at 65°C; for paper filters: 50% formamide–5× SSC–0.1% SDS; rinse in 5× SSC–0.1% SDS at 65°C). After autoradiography, the probes were stripped from nylon filters by boiling in 0.01× SSC–1% SDS for 20 min and from paper filters by washing in 0.5 N NaOH and then 2× SSC–0.1% SDS at room temperature for 20 min each. Repeat hybridization with the plasmid DNA filters and the aerobactin probe, after hybridization with and stripping of the other probes, demonstrated minimal loss of plasmid DNA from the filters during processing.

Colony hybridization. Colonies grown overnight on Luria agar (23) were transferred to paper filters (Whatman 541; Whatman Ltd., Maidstone, England) for hybridization as previously described (22).

Determination of phenotypic expression of adhesins, hemolysin, and aerobactin. Isolates were passed daily on 5% sheep blood agar and in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C for 21 days. Agglutination tests to detect mannose-specific binding (mediated by type 1 fimbriae) and galactose-galactose-specific binding (mediated by P fimbriae) were performed initially and every 7 days during serial passage, as previously described (15). Blood agar plates were inspected daily for 4 days during serial passage for evidence of hemolysis.

Aerobactin production was determined during daily passage on supplemented M9 agar (23) containing 0.2 mM 2,2′-dipyridyl (Sigma Chemical Co., St. Louis, Mo.) by using a cross-feeding bioassay with the aerobactin-requiring strain LG1522 (generously provided by P. Williams, Leicester, England), as previously described (6, 7).

Aerobactin receptor expression was assessed by using an aerobactin utilization assay, as previously described (35), with modifications. Test strains grown overnight in Luria broth (23) were added to M9 agar containing 0.2% Casamino Acids (Difco) and 0.3 mM 2,2′-dipyridyl at 45°C to give a concentration of 10^3 organisms per ml, and this mixture was poured into sterile culture plates. Two sterile filter paper disks were placed on the agar surface of each pour plate: one was inoculated with 10 μl of sterile supernatant from the aerobactin-producing, enterobactin-deficient strain LG1315 (36) grown at 37°C for 48 h in supplemented M9 broth plus 0.2 mM 2,2′-dipyridyl, and the other was inoculated with 10 μl of 0.1 mM $FeCl_3$ in 0.05 N HCl. Utilization of aerobactin was indicated by a zone of enhanced growth around the disk containing the LG1315 supernatant. (P. Williams generously provided strain LG1315.)

Determination of antibiotic susceptibility. Susceptibility of the strains to 12 antimicrobial agents was determined by a disk diffusion technique. Strains which had zones of inhibition corresponding to resistance or intermediate susceptibility to an antimicrobial agent by National Committee for Clinical Laboratory Standards criteria were considered to be resistant to that agent.

Mating experiments. Mating experiments were performed in broth as previously described (2). The four urosepsis strains which had plasmid-encoded aerobactin (see below), a single plasmid, and resistance to multiple antimicrobial agents (including tetracycline) were each mated with recipient strain K-12 395-1, which has no plasmids, is Nal^r, and carries a *lac* mutation (29). Transconjugants were selected on Luria agar (23) (supplemented with 50 μg of nalidixic acid per ml and 20 μg of tetracycline per ml) and tested along with the recipient strain for antimicrobial-agent resistance, lactose fermentation, and aerobactin production. Plasmid DNA was isolated from the transconjugants by the procedure of Kado and Liu (16).

Statistical methods. Statistical comparisons were made and associations in categorical data were examined by determining differences in proportion, using the chi-square statistic or Fisher's exact test. The presence or absence of characteristics in ranked groupings was analyzed by using the chi-square statistic for testing for a linear trend in proportions (8). The rank-sum test was used to determine differences in ordinal data between groups (9).

RESULTS

Characteristics of patients. Fifty-eight patients with urosepsis were studied. Compromising medical or urological conditions were present singly or in combination in 47 (81%) of the patients, including urinary tract abnormalities in 31 (53%), urinary tract instrumentation in 16 (28%), and medical illnesses in 26 (45%). Eleven (19%) patients had none of these conditions and were considered noncompromised.

Colony hybridization and phenotypic analysis of strains. One blood isolate from each patient was examined for the presence of the virulence factor determinants (by using colony hybridization) and for phenotypic expression of the virulence factors (Table 1).

The aerobactin probe detected 45 (78%) of the strains, of which all demonstrated at least partial functioning of the aerobactin system. Of these 45 strains, 38 produced aerobactin and all but one (which was an aerobactin-producing strain) expressed the aerobactin receptor. Of the 13 strains not detected with the probe (probe-negative strains), 3 demonstrated partial functioning of the aerobactin system: 2 were positive in the aerobactin production assay (but negative in the receptor assay), and 1 was positive in the receptor assay (but negative in the production assay). Passage on low-iron agar for 14 days stimulated aerobactin production in only 2 of the 20 strains which did not produce aerobactin initially (Fig. 1).

The P fimbria probe detected 43 (74%) of the strains, and 36 (62%) of the strains were positive for P fimbriae in the phenotype assay. All phenotypically positive strains were positive on initial testing; there was no increased detection of P fimbriae with serial agar passage (Fig. 1). Passage in broth inhibited expression of P fimbriae. All P fimbria-expressing strains were detected with the P fimbria probe.

The hemolysin probe detected 27 (47%) of the strains, and 26 (45%) of the strains were hemolytic on blood agar. One

TABLE 1. Results of phenotype and genotype testing for virulence factors among 58 *E. coli* isolates from patients with urosepsis

Virulence factor	No. (%) of strains positive for virulence factor			
	Phenotype testing	Colony hybridization	Southern hybridization	
			Total DNA	Plasmid DNA
Aerobactin system	45 (78)	45 (78)	45 (78)	12 (21)
Production	40[a] (69)			
Utilization	45 (78)			
P fimbriae	36[a] (62)	43 (74)	44 (76)	0 (0)
Hemolysin[b]	26 (45)	27 (47)	25 (43)	0 (0)
Type 1 fimbriae	51[a](88)	57 (98)	57 (98)	0 (0)

[a] Total number of strains positive for virulence factor production after serial passage for 21 days (on agar, for P fimbriae; in broth, for type 1 fimbriae) or 14 days (on low-iron agar, for aerobactin).

[b] One hemolytic strain was not detected by hybridization assays; two nonhemolytic strains were positive by colony hybridization but differed from hemolytic strains by Southern hybridization (see the text).

hemolytic strain was not detected with the probe, and two nonhemolytic strains were positive in the hybridization assay. Passage of the strains did not affect hemolysin expression (Fig. 1).

The type 1 fimbria probe detected 57 (98%) of the strains, and 51 (88%) were positive in the phenotype assay. Repeated passage in broth was required for expression of type 1 fimbriae by most strains (Fig. 1). Because of the high prevalence of the type 1 fimbria determinants among the strains, associations between type 1 fimbriae and other bacterial or host characteristics were not pursued.

Resistance to one or more antimicrobial agents was found in 30 (52%) of the strains. The number of antimicrobial agents (of 12 tested) to which these 30 strains were resistant ranged from 1 to 10, with a median of 3.5.

Southern blot analysis of plasmid DNA. To determine the plasmid-versus-chromosomal location of the virulence factor determinants, Southern blots of plasmid DNA from all 58 strains were hybridized with each of the probes (Fig. 2 and Table 1). The aerobactin probe hybridized with a single plasmid in 12 (21%) of the strains. Plasmids hybridizing with the probe ranged in size from 70,000 to 90,000 daltons. All strains with plasmid-encoded aerobactin (plasmid aerobactin) were positive in phenotype assays for aerobactin production and aerobactin receptor function. No plasmids were detected with the probes for P fimbriae, hemolysin, or type 1 fimbriae.

Southern blot analysis of total DNA. Total DNA was isolated from each strain and subjected to four different endonuclease digestions: *Hin*dIII-*Pst*I, *Sal*I, *Eco*RI, and *Bam*HI. The digested DNA was subjected to Southern blot analysis with each of the virulence factor probes.

(i) Aerobactin. The 2-kb *Hin*dIII-*Pst*I aerobactin probe detected a single corresponding 2-kb *Hin*dIII-*Pst*I fragment in each probe-positive strain. Single fragments of various sizes were detected in the *Sal*I, *Bam*HI, and *Eco*RI digests of all except one of the probe-positive strains. In this strain, two *Sal*I, *Bam*HI, and *Eco*RI fragments were detected, suggesting that it may contain two copies of the aerobactin determinants (Fig. 3). One copy of the aerobactin determinants was shown to be plasmid associated in this strain, but it was not determined whether both copies were plasmid associated or whether one was chromosomal.

(ii) P fimbriae. Of the 43 P fimbria probe-positive strains, 7 appeared to have two copies of the P fimbria determinants, as evidenced by detection of homology with at least two fragments in two or more of the restricted total DNA preparations from these strains (including the *Hin*dIII-*Pst*I and *Eco*RI digests for two strains, the *Hin*dIII-*Pst*I and *Sal*I digests for one strain, the *Hin*dIII-*Pst*I, *Sal*I, and *Eco*RI digests for one strain, the *Hin*dIII-*Pst*I, *Eco*RI, and *Bam*HI digests for one strain, and all four digests for two strains [Fig. 3]). One strain which was repeatedly negative with the P fimbria probe by colony hybridization was strongly positive by Southern hybridization for all four restricted total DNA preparations. This strain was classified as probe positive in subsequent analyses.

(iii) Hemolysin. Of the 25 hemolysin probe-positive strains, 2 had an extra band in the *Eco*RI digest only, indicating the presence of an additional recognition site for this enzyme within the *hlyA* gene in these strains, but neither appeared to have more than one copy of this gene, since other restriction digests showed only the expected number of bands. The two strains which were probe positive, by colony hybridization but nonhemolytic showed homology to the hemolysin probe by Southern hybridization in fragments which were larger than expected for the *hlyA* gene and gave much weaker autoradiographic signals than were seen with the hemolytic strains. These strains were classified as Southern blot negative for hemolysin in subsequent analyses.

(iv) Type 1 fimbriae. Of the 57 type 1 fimbria probe-positive strains, 6 had two bands in the *Hin*dIII-*Pst*I digest (one band was expected), indicating acquisition of a recognition site for one of these enzymes within the homologous sequence, but none appeared to have more than one copy of the type 1 fimbria determinants, since other restriction digests showed only the expected number of bands.

Aerobactin phenotype and genotype, in comparison with other bacterial and host characteristics. There was no evi-

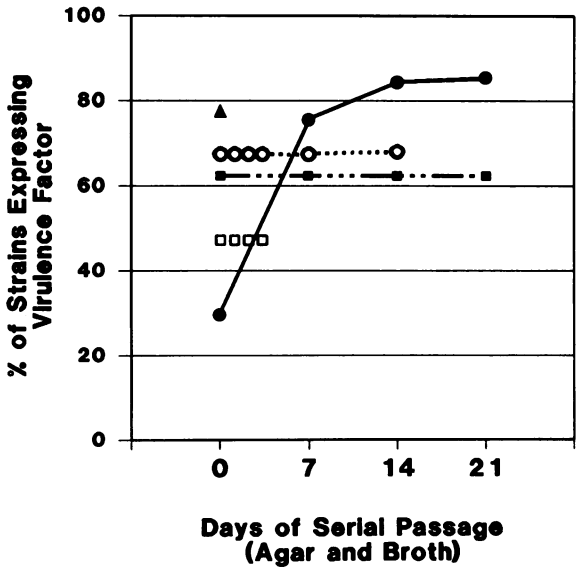

FIG. 1. Percentage of strains expressing virulence factors when tested repeatedly during serial passage. The results shown are from passage on 5% sheep blood agar (P fimbriae [■] and hemolysin [□]), on supplemented M9 agar plus 2,2'-dipyridyl (aerobactin production [○]), in Luria broth (aerobactin utilization [▲]), or in brain heart infusion broth (type 1 fimbriae [●]). The symbols indicate the day(s) on which virulence factor tests were performed.

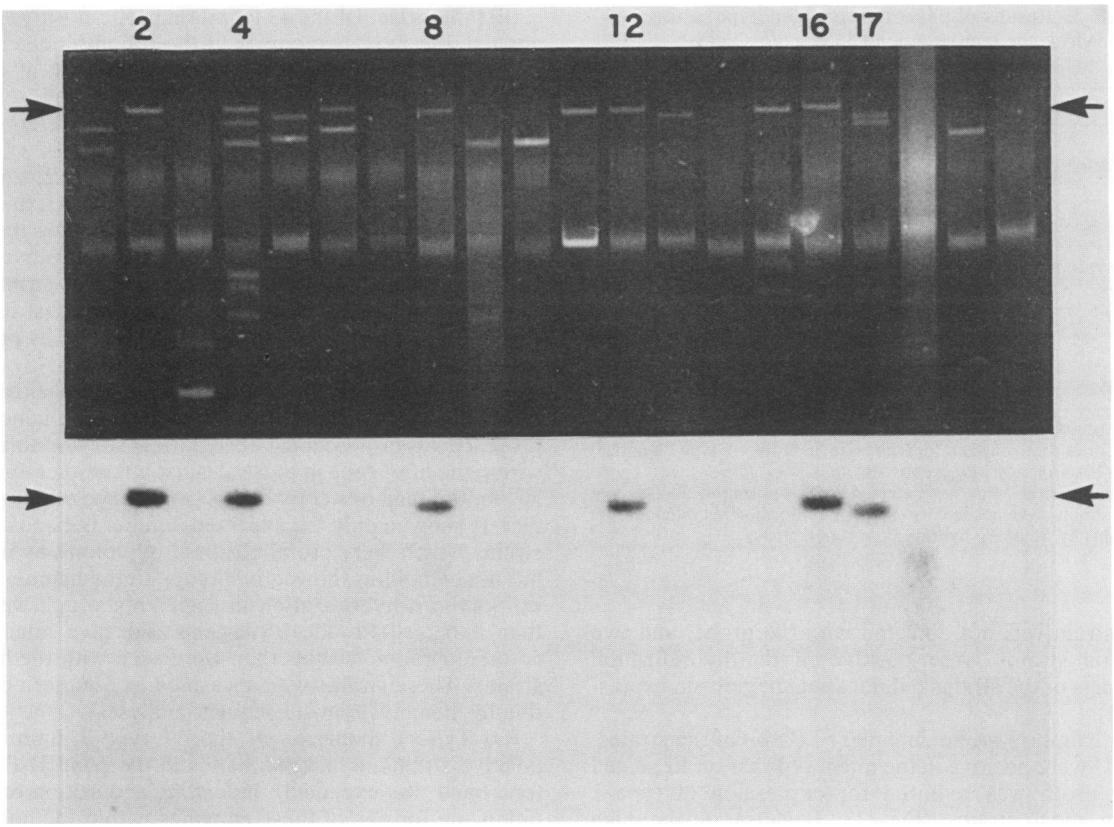

FIG. 2. Plasmid profiles and hybridizations with aerobactin probe. (Top) Plasmid profile of strains 1 to 20. The arrows indicate the positions of plasmids corresponding to signals on the autoradiograph (bottom). (Bottom) Autoradiograph from hybridization of plasmid DNA from strains 1 to 20 with the 2-kb *Hin*dIII-*Pst*I fragment of pJHC-V12 (aerobactin probe; see the text). Aerobactin determinants (arrows) were identified on large plasmids in strains 2, 4, 8, 12, 16, and 17. The lanes are positioned below the corresponding lanes in the top panel for visual reference.

dence for an association between the presence of or expression of the aerobactin determinants and P fimbriae, hemolysin, antimicrobial-agent resistance, or compromising medical or urological conditions. In contrast, striking associations between the aerobactin determinants and other bacterial or host characteristics were seen when strains with plasmid aerobactin were considered separately from strains with chromosomally encoded (chromosomal) aerobactin only. Plasmid aerobactin was found only in strains from compromised hosts, of whom 26% had strains with plasmid aerobactin, whereas chromosomal aerobactin accounted for all of the aerobactin-positive strains from the noncompromised patients (Table 2). In contrast to chromosomal aerobactin, plasmid aerobactin was associated with strains lacking either the P fimbria determinants or the hemolysin determinants (Table 2). In addition, plasmid aerobactin was strikingly associated with an increased prevalence and extent of antimicrobial-agent resistance, whereas chromosomal aerobactin was associated with an absence of antimicrobial-agent resistance (Table 2). Plasmid aerobactin was independently associated with resistance to certain antimicrobial agents, including streptomycin, tetracycline, ka-

TABLE 2. Comparison of aerobactin genotype with other bacterial and host characteristics

Characteristic	No. (%) of strains positive for characteristic					
	Aerobactin determinants in total DNA[a]			Location of aerobactin determinants		
	Absent (n = 13)	Present (n = 45)	P value[b]	Plasmid (n = 12)	Chromosome[c] (n = 33)	P value[b]
Resistance to ≥1 antimicrobial agent	7 (54)	23 (51)	NS	11 (92)	12 (36)	0.001
Antimicrobial-agent resistance score[d]	1	1	NS	4.5	0	0.0001
P fimbriae[a]	10 (77)	34 (76)	NS	6 (50)	28 (85)	0.004
Hemolysin[a]	6 (46)	19 (42)	NS	0 (0)	19 (58)	0.0004
Compromising medical or urological condition	11 (85)	36 (80)	NS	12 (100)	24 (73)	0.09

[a] Based on Southern blot analysis of total DNA with probe for aerobactin, P fimbriae, or hemolysin.
[b] P values were calculated by using the chi-square statistic or Fisher's exact test for comparisons between the two groups. NS, Not significant.
[c] Total-DNA positive but plasmid-DNA negative with aerobactin probe.
[d] Median number of antimicrobial agents (of 12 tested) to which the strains were resistant.

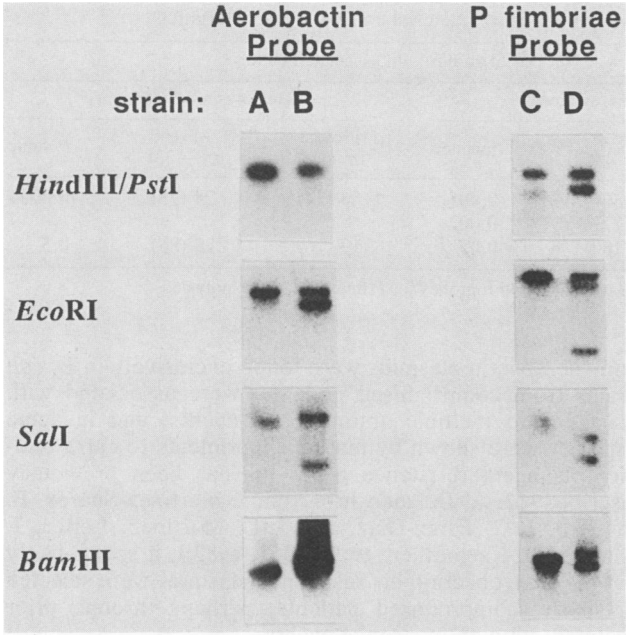

FIG. 3. Evidence of multiple copies of virulence factor determinants. Autoradiographs from Southern hybridization of restricted total DNA with the probe for aerobactin (strains A and B) or for P fimbriae (strains C and D) showed a single homologous band in each of four restricted DNA preparations (identified on the left) for strains A and C. In contrast, multiple bands were identified in two or more preparations of restricted total DNA from strains B and D.

namycin, sulfisoxazole, and ampicillin ($P < 0.05$ for each agent).

Mating experiments confirmed that antimicrobial-agent resistance genes were carried on aerobactin plasmids. The four strains which had a single plasmid, multiple antimicrobial-agent resistance, and plasmid aerobactin were each separately mated with the recipient strain K-12 395-1 (29). Each resulting transconjugant contained a single plasmid of the same apparent molecular size as that in the corresponding donor strain. Transconjugant strains produced aerobactin and had patterns of multiple antimicrobial-agent resistance similar to those of the corresponding donor strains while retaining the genomic traits of the recipient strain (i.e., a *lac* mutation and Nal^r).

P fimbria and hemolysin genotypes and phenotypes. The absence of determinants for P fimbriae was significantly associated with compromised patients (Table 3). Within the subgroups of patient abnormalities, the P fimbria determinants were less frequent in strains from patients with urinary tract abnormalities (present in 65% of patients with and 89% of those without an abnormality; $P = 0.04$), and a trend of similar magnitude was seen in association with urinary tract instrumentation. In contrast, no association was apparent between the P fimbria determinants and medical illnesses (present in 73% of patients with and 78% of those without an illness). The eight strains which were probe positive but phenotype negative for P fimbriae were all from compromised patients.

The presence of the P fimbria determinants was also significantly associated with a lack of antimicrobial-agent resistance, as was the presence of the hemolysin determinants (Table 3). In addition, a strong association was seen between the presence of the P fimbria determinants and the presence of the hemolysin determinants (Table 3).

Combined CVF genotype in comparison with other bacterial and host characteristics. Since P fimbriae, hemolysin, and chromosomal aerobactin are all chromosomally encoded virulence factors (CVFs) which might be conserved together in uropathogenic strains, their presence in combination was assessed and compared with other bacterial and host factors (Fig. 4).

Nineteen (33%) strains had all three CVF determinants (P fimbriae, hemolysin, and chromosomal aerobactin). Fourteen (24%) strains had two CVFs, 17 (29%) had only one, and 8 (14%) had none. Strains lacking these CVFs were associated with increased antimicrobial-agent resistance ($P = 0.0003$), plasmid aerobactin ($P = 0.0001$), and compromised patients ($P = 0.06$) more commonly than were strains with multiple CVFs; an increase in the number of CVFs was associated with a progressive decrease in the frequency of these associated characteristics (Fig. 4). The negative association between combined CVFs and compromised hosts appeared to be due not to selection for CVF-deficient strains in the compromised patients but rather to an association between noncompromised patients and strains with multiple CVFs (Fig. 5). In addition, seven (21%) of the strains with two or three CVFs appeared to have more than one copy of the aerobactin or P fimbria determinants, a phenomenon seen in only one (4%) of the strains with no CVF or one CVF.

DISCUSSION

Aerobactin is a bacterial iron sequestration and transport system which enables *E. coli* to grow in iron-poor environments such as dilute urine and complement-depleted serum

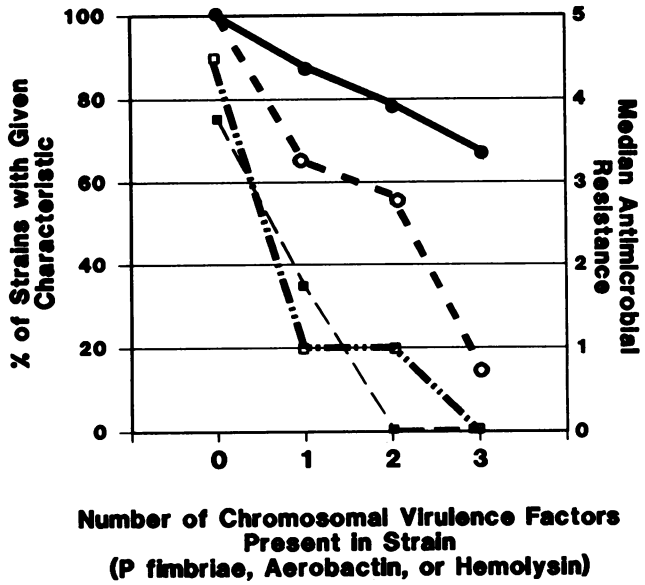

FIG. 4. Number of CVFs versus other bacterial characteristics. Strains were sorted by the number of different CVF determinants present (including P fimbriae, hemolysin, and chromosomal aerobactin, as determined by Southern hybridization of total and plasmid DNA with the appropriate probes). The prevalence of associated characteristics (left *y* axis) and median number of antimicrobial agents to which the strains were resistant (right *y* axis) (□) was determined for strains with 0, 1, 2, and 3 different CVF determinants (*x* axis). Symbols for left *y* axis: ●, compromised patient; ○, resistance to one or more antimicrobial agents; ■, plasmid aerobactin.

TABLE 3. Comparison of P fimbria and hemolysin genotypes with other bacterial and host characteristics

Characteristic	No. (%) of strains with associated factor					
	P fimbria determinants[a]			Hemolysin determinants[a]		
	Present (n = 44)	Absent (n = 14)	P value	Present (n = 25)	Absent (n = 33)	P value
Resistance to ≥1 antimicrobial agent	18 (41)	12 (86)	0.005	6 (24)	24 (73)	0.0004
Hemolysin[a]	24 (55)	1 (7)	0.002			
Compromising medical or urological condition	33 (75)	14 (100)	0.05	19 (76)	28 (85)	0.5

[a] P fimbria and hemolysin genotypes were determined by Southern blot analysis of total DNA with probes for P fimbriae and hemolysin.

(25). P fimbriae mediate bacterial adherence to human epithelial cells via digalactoside-specific binding to the P-blood-group antigens, which are expressed throughout the urinary tract and facilitate ascending infection of the ureter and kidney (15). Hemolysin is associated with necrotoxicity in rabbits and cytotoxicity in tissue culture (24) and promotes microbial growth by releasing iron from lysed erythrocytes (18). Each of these virulence factors is produced by a greater fraction of strains causing invasive infections, such as pyelonephritis and bacteremia, than by fecal strains (5, 15, 24, 25, 27, 32, 33). We identified aerobactin determinants in 78% of *E. coli* strains isolated from the blood of patients with urosepsis. This frequency exceeds that of the hemolysin determinants (43%) and equals that of the P fimbria determinants (74%) in these urosepsis strains and is similar to the frequency of aerobactin (60 to 75%) reported among *E. coli* isolates causing other types of invasive infections (5, 25).

Aerobactin has been recognized as a plasmid-associated virulence factor in *E. coli* for some time and accounts for some of the virulence properties conferred by the ColV plasmid (36). We found aerobactin determinants to be plasmid associated in 27% of aerobactin-positive strains and chromosomal in 73%, a result similar to that described for *E. coli* strains which cause neonatal sepsis (35). Since in our study aerobactin plasmids were found exclusively in *E. coli* strains from compromised patients, were associated with resistance to multiple antimicrobial agents, and in some instances were shown by mating experiments to carry antimicrobial-agent resistance genes (as has been previously described [12; A. Delgado-Iribarren, J. Martinez-Suarez, F. Baquero, J. C. Perez-Diaz, and J. L. Martinez, Letter, J. Antimicrob. Chemother. **19**:552–553, 1987]), it seems likely that these aerobactin-plus-resistance plasmids were selected for in the compromised patients, perhaps through prior treatment with antimicrobial agents.

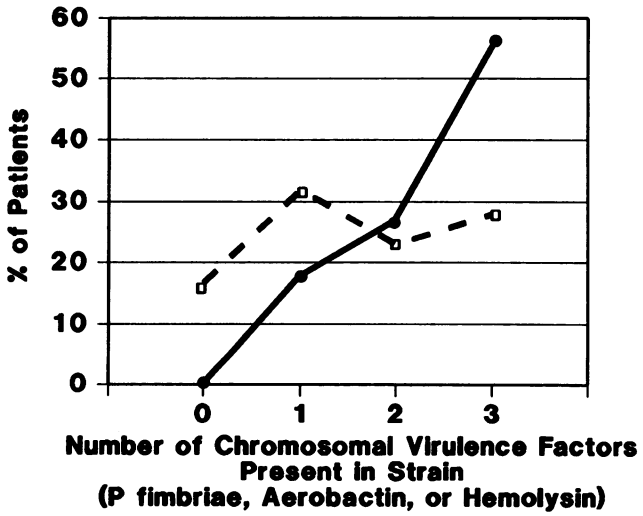

FIG. 5. Distribution of combined CVFs in strains from compromised and noncompromised patients. Patients were sorted by the presence or absence of compromising medical and urological conditions and further sorted by the number of CVF determinants present in the strains isolated from them (as described in the legend to Fig. 4). The fraction of each group of patients (compromised [□] [n = 47] or noncompromised [●] [n = 11]) associated with each CVF category (0 to 3) is shown.

In contrast to plasmid aerobactin, we found that chromosomal aerobactin was associated with noncompromised patients and with the determinants for P fimbriae and hemolysin. Since P fimbriae also were associated with noncompromised patients and since the genes for P fimbriae and hemolysin were chromosomal in all instances, it is reasonable to speculate that chromosomal aerobactin is part of the group of conserved CVFs which facilitate invasive urinary tract infections in noncompromised hosts (33). The genetic basis for a linkage between virulence genes in strains of uropathogenic *E. coli* has previously been demonstrated for the P fimbria and hemolysin genes (17, 21, 31); it remains to be shown whether the statistical associations we found between CVF determinants are due to their proximity on the chromosome. In support of the hypothesis that these three CVFs together constitute a highly uropathogenic genotype is our finding that strains with the greatest number of CVFs predominated among the noncompromised patients.

In contrast to the aerobactin system, we found no evidence of plasmid-associated genes for P fimbriae, hemolysin, or type 1 fimbriae. The chromosomal-versus-plasmid location of the determinants for P fimbriae and type 1 fimbriae had not been extensively studied heretofore, although the position of these genes has been mapped on the *E. coli* chromosome of a urinary tract isolate (13). The alpha-hemolysin determinant has been reported to be plasmid associated in animal diarrheal disease isolates (14), but in a limited survey of strains from human extraintestinal *E. coli* infections the hemolysin determinants appeared to be chromosomal (24). Our findings are consistent with these reports and demonstrate that in *E. coli* strains causing urosepsis, P fimbriae, type 1 fimbriae, and hemolysin are chromosomally encoded.

The presence of multiple copies of the determinants for aerobactin or P fimbriae in a number of the urosepsis strains was suggested by hybridization of the corresponding DNA probe with more than the expected number of fragments in two or more preparations of restricted total DNA from these strains. Multiple copies of the determinants for P fimbriae or hemolysin in uropathogenic *E. coli* strains have been previously described (13, 17, 31). The trend we identified toward an association between combined CVF determinants (aero-

bactin, P fimbriae, and hemolysin) and multiple copies of one of these determinants is intriguing and suggests the possibility of a selective advantage in invading noncompromised hosts for strains with a greater complement of CVF genes.

A comparison of phenotypes and genotypes for aerobactin, P fimbriae, type 1 fimbriae, and hemolysin showed that all strains with hemolysin determinants produced hemolysin but that a number of strains which had sequence homology with the aerobactin, P fimbria, or type 1 fimbria probe did not produce the corresponding virulence factor. The failure of seven (12%) of the aerobactin probe-positive strains to produce aerobactin is consistent with the proportion (8%) of probe-positive, phenotype-negative strains among the clinical isolates studied by Carbonetti et al. (5) and may be attributable to persistence of sufficient intracellular iron to prevent expression of the aerobactin operon. However, in our strains the absence of detectable aerobactin production despite serial passage for 14 days under low-iron conditions and the presence of aerobactin receptor function suggests instead a defect in siderophore biosynthesis. The two strains (3.4%) which lacked aerobactin determinants by Southern and colony hybridization but which supported the growth of the indicator strain in the bioassay for aerobactin may have been producing a siderophore other than aerobactin, as suggested by Carbonetti et al. (5) to explain this phenomenon, which they encountered in 2.3% of the human isolates they studied. However, Linggood et al. (19) demonstrated that the siderophore produced by certain animal isolates which did not hybridize with probes prepared from the prototype pColV-K30 aerobactin determinants yet which supported the growth of strain LG1522 was actually aerobactin.

The discordant fimbrial phenotype and genotype testing results are of interest in that O'Hanley et al. (27) were able to demonstrate appropriate agglutination reactions in 100% of urinary tract infection strains which were positive with the P fimbria or type 1 fimbria probe, although passage for up to 18 days was required to induce fimbrial expression in some strains. The absence of strains with discrepant genotypes and phenotypes in their study, in contrast to our findings, may have been due to the absence of patients with urosepsis or compromising medical or urological conditions, in contrast to the population we investigated. There is little reason to suspect that the conditions we used to induce fimbrial expression would fail to reverse whatever inhibition of fimbrial production might have resulted from the manipulations involved in isolating and stocking the strains in the clinical microbiology laboratories; thus, it seems likely that the probe-positive, phenotype-negative strains were also phenotype negative in vivo. That all such functionally nonfimbriated strains came from compromised patients is consistent with the concept that fimbriation is not necessary for urosepsis in compromised hosts (15).

In the present study, all strains which expressed P fimbriae did so when first tested, in contrast to the progressive rise in the P-fimbriated fraction described by O'Hanley et al. (27). These discrepant findings may be due to the different bacterial populations studied, in that our urosepsis strains were isolated from blood, in which selective pressures from phagocytes would favor strains expressing P fimbriae, which have been shown to be antiphagocytic (1, 10).

The progressive rise we observed in the fraction of strains expressing type 1 fimbriae during serial passage, also noted by O'Hanley et al. (27), may have been due to an initial inhibition of fimbrial expression by the multiple passages on agar involved in isolating and stocking these organisms. Enhanced phagocytosis of bacteria expressing type 1 fimbriae (1, 10, 30) could select for the nonfimbriated state in vivo in patients with bacteremia, which also could contribute to the initial low level of type 1 fimbriation.

The absence of detectable sequence homology between the hemolysin probe and DNA from one of the hemolytic strains suggests that in this strain a functional hemolysin may be encoded by genes which differ substantially from the alpha-hemolysin genes found in the other hemolytic strains.

In any case, it appears that phenotypes and genotypes for aerobactin, adhesins, and hemolysin are frequently and variably discordant, that aerobactin and adhesin phenotypes vary with testing conditions, and that depending on which assays are used (i.e., virulence factor production or DNA hybridization), different characteristics of the organism will be detected. These observations suggest that in choosing tests and conditions to detect virulence factors, consideration must be given to whether the genotype or phenotype is of greater interest.

In conclusion, we determined that among *E. coli* strains causing urosepsis, aerobactin determinants were as prevalent as genes for other recognized uropathogenic virulence factors. Aerobactin determinants were more commonly detected on the chromosome than on plasmids. Some aerobactin plasmids carried antimicrobial-agent resistance genes, possibly explaining the observed association between plasmid aerobactin, antimicrobial-agent resistance, and patients with compromising urological or medical conditions. We propose that chromosomal aerobactin, P fimbriae, and hemolysin together constitute a uropathogenic genomic configuration which is characteristic of the antimicrobial-agent-susceptible *E. coli* strains causing urosepsis in noncompromised patients.

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