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Aerobactin-Mediated Iron Uptake by *Escherichia coli* Isolates from Human Extraintestinal Infections

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A total of 516 strains of *Escherichia coli* were screened for the presence and expression of the aerobactin iron uptake system. The incidence was markedly higher among clinical isolates from patients with septicemia (68.8%), pyelonephritis (74.6%), and symptomatic (59.8%) and asymptomatic (63.2%) lower urinary tract infections than among normal human fecal isolates (34.3%).

The aerobactin-mediated iron uptake system of *Escherichia coli* plasmid ColV-K30 (20) confers a significant selective advantage for bacterial growth in conditions of iron stress, such as those prevailing in the body of an experimentally infected animal (21, 22). The clinical importance of the aerobactin system, however, and its ubiquity among pathogenic isolates of *E. coli* have not been systematically assessed, although small-scale studies have suggested a high incidence among strains isolated from the blood of patients with various diseases (11, 16). In this paper we present the results of the first extensive systematic survey of clinical *E. coli* isolates. We show a significantly higher incidence of the aerobactin system among isolates from patients with septicemia and also from other human extraintestinal infections, particularly pyelonephritis and meningitis, than among the normal fecal flora of healthy females; we propose, therefore, that a crucial aspect of the virulence of such isolates is their ability to acquire iron from high concentrations of transferrin and lactoferrin in body fluids and secretions.

A total of 516 *E. coli* strains, of which 286 were isolated in Finland and 230 were isolated in England, were analyzed. Of the Finnish strains, 180 were isolated between 1977 and 1981 from the urine of young girls (mean age, 6.4 years) with various levels of urinary tract infections (UTI) (71 from patients with pyelonephritis, 55 from patients with cystitis, and 54 from patients with asymptomatic bacteriuria), and 45 were isolated from the feces of healthy children. Patient criteria (6, 18) and a full characterization (18, 19) of these strains have been reported previously. A total of 61 of the Finnish strains were isolated between 1974 and 1977 from the blood or cerebrospinal fluid of infants who were less that 5 months old (43 from patients younger than 21 days) and had septic infections. These strains also have been characterized previously in detail (9). Of the English strains, 176 were isolated between 1980 and 1982 from adult females between the ages of 18 and 45. Of these, 154 were isolated from patients with acute lower UTI, and 22 were isolated from pregnant women with asymptomatic bacteriuria (13). A further 32 strains were isolated from the blood of a group of hospital patients with septicemia, and 22 strains were isolated from the feces of a group of healthy young women.

The strains were screened with two tests. The genetic determinants of the aerobactin iron uptake system of plasmid ColV-K30 were previously cloned as the multicopy recombinant plasmid pABN1 (1) and mapped (3), and we used restriction fragments of this plasmid as probes in colony hybridization (7) to detect homologous nucleotide sequences among the clinical isolates. The probes, which were labeled with ^{32}P by nick translation (15), were a 2-kilobase *Aval* fragment from within the aerobactin biosynthesis region and a 2.3-kilobase *Pvu*II fragment containing most of the ferric aerobactin receptor gene (Fig. 1); the two probes gave identical results. In addition, we used a bioassay for the aerobactin system that uses an *E. coli* K-12 strain, designated LG1522 (*fepA* ColV-K30 *iuc*), which can grow in iron-restricted conditions if provided with aerobactin but not enterochelin (4). Table 1 shows the number and percentage of isolates in each diagnostic group that were positive in the two tests. Data obtained with English and Finnish strains were pooled because statistical analysis demonstrated that there was no significant difference in the incidence of aerobactin genes in strains within a diagnostic group.

Note that although hybridization directly detects the presence of particular genes, the biological assay depends on the expression of the aerobactin system. Agreement between the two methods of screening was found to be reasonably high; of the 516 isolates tested, 258 (50%) were positive in both tests, and 203 (39.3%) were negative in both tests. Nevertheless, the fact that a significant number of strains showed disagreement emphasizes the complementary nature of the two tests and the desirability of performing both if possible. For example, 43 strains were positive in the hybridization study but negative in the bioassay for aerobactin; we presume that this is due simply to a failure to induce the expression of the aerobactin system, since we observed that extensive serial passaging through low-iron medium may in some cases be necessary to deplete intracellular stores of iron. More interestingly, 12 strains were positive in the bioassay and yet did not hybridize to the two defined aerobactin gene probes. To rule out the possibility that hybridization may have been inhibited by the presence of, for instance, capsular material, we repeated hybridization studies with purified DNA samples of these strains, with identical results. It may be that they produce another

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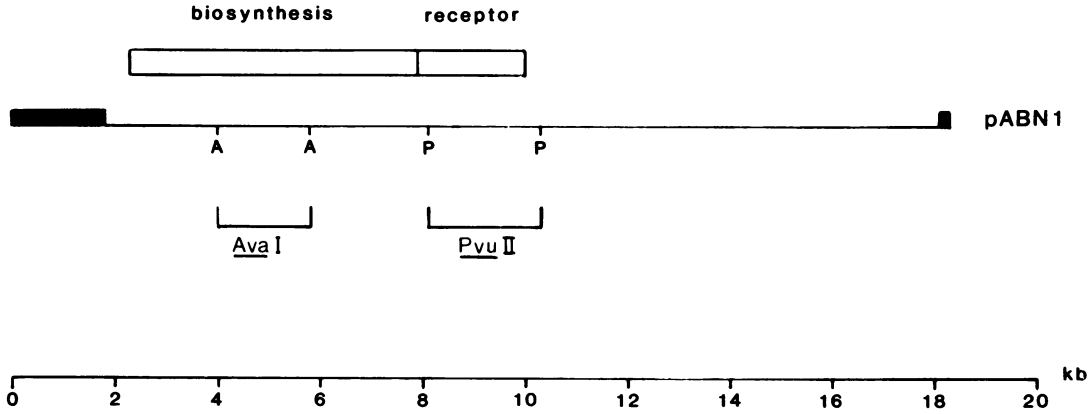

FIG. 1. Iron uptake region of plasmid pABN1 and restriction fragments used as probes for colony hybridization. The solid bars represent the DNA of the vector plasmid, and the thin line represents a 16-kilobase-pair (kb) *Hin*dIII-generated restriction fragment from plasmid ColV-K30 which carries the entire aerobactin system (1). Regions involved in the biosynthesis of aerobactin and the synthesis of the ferric aerobactin receptor protein (3) are indicated. Only the *Ava*I (A) and *Pvu*II (P) endonuclease cleavage sites relevant to the preparation of the two probes are shown. Fuller restriction maps of plasmid pABN1 have been published elsewhere (1, 3).

siderophore that can be utilized by the indicator strain LG1522, which would suggest that the receptor protein may not be totally specific. Alternatively, they may synthesize the same siderophore by a different pathway. We are currently investigating this small group of strains to determine the nature and significance of the discrepancy.

The frequency of positive strains was highest among strains isolated from patients with pyelonephritis (74.6%) and septic infections (68.8%) and slightly lower among strains isolated from patients with symptomatic lower UTI (59.8%) and asymptomatic bacteriuria (63.2%). In contrast, only about one-third of normal fecal isolates carried the genes for the aerobactin system, a significantly lower incidence (at the 5% level in a two-way χ^2 test) than in any of the other diagnostic groups. It is interesting that among the Finnish isolates from patients with septic infections, for which the most detailed patient case histories were known, there was no particular association between the onset or fatality of the infection and the occurrence of the aerobactin system among the isolates. That is, similar proportions of strains from patients younger than 21 days (27 of 43, 62.8%) and of strains from patients 22 days to 5 months old (12 of 18, 66.7%) were positive (in both of the test), as were 6 of the strains from 10 patients who died. Furthermore, of the strains isolated in Finland from patients with septic infections, 13 were from patients with identified meningitis; 10 (76.9%) of these were found to carry the aerobactin system.

It is known that high dietary iron levels increase the susceptibility of rats to experimental pyelonephritis (8). Also, iron-regulated outer membrane proteins have been shown to be expressed by enteric bacteria collected directly from the urine of patients with UTI (10). In considering the role of the aerobactin system in the pathogenesis of UTI, it is important to note that its incidence was actually slightly higher in asymptomatic isolates from the lower urinary tract than in cystitis isolates, suggesting that efficient iron sequestration is necessary for an organism to multiply within the iron-restricted conditions of the urinary tract but that other virulence factors are required to determine full pathogenicity. Such factors may include mannose-resistant adhesins (12, 14), which are more prevalent in *E. coli* isolated from women with cystitis than in strains isolated from women with asymptomatic infections. Of particular interest also is the high incidence of the aerobactin system (74.6%) among isolates from patients with pyelonephritis, as high as that of P fimbriae (76% for the same set of pyelonephritis-associated strains) and higher than that of hemolytic activity (60% for the same strains) (19), both of which have previously been recognized as bacterial virulence-enhancing factors in UTI (2, 5, 17). These findings confirm that bacteria are able to

TABLE 1. Incidence of the aerobactin system among *E. coli* isolates of different diagnostic groups

Source	No. of strains	No. (%) of strains positive:			
		In both tests[a]	In colony hybridization only	In bioassay only[b]	Total
Pyelonephritis	71	45 (63.4)	8 (11.3)	0	53 (74.6)
Symptomatic lower UTI (cystitis)	209	100 (47.8)	18 (8.6)	7 (3.3)	125 (59.8)
Asymptomatic bacteriuria	76	40 (52.6)	6 (7.9)	2 (2.6)	48 (63.2)
Septic infections	93	57 (61.3)	4 (4.3)	3 (3.2)	64 (68.8)
Normal fecal isolates	67	16 (23.9)	7 (10.4)	0	23 (34.3)
Total	516	258 (50.0)	43 (8.3)	12 (2.3)	313 (60.7)

[a] Strains were tested by colony hybridization with the probes described in the text and with a final posthybridization wash in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and by a bioassay with strain LG1552.

[b] In subsequent tests, strains of this type were found to show no hybridization even at a lower stringency (up to 1× SSC) and to be unable to cross-feed a strain lacking the aerobactin receptor.

grow in the iron-restricted onditions of the urinary tract and emphasize a potential role for aerobactin-mediated iron uptake in the pathogenesis of UTI by *E. coli*.

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