Virulence-Related Genes in ColV Plasmids of *Escherichia coli* Isolated from Human Blood and Intestines

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DNA probes for the colicin V, *traT*, *iss*, and *iu* genes were used in this study of four representative ColV plasmids together with 200 *Escherichia coli* strains isolated from the stools of patients with diarrhea and 146 *E. coli* strains isolated from the blood of patients with bacteremia. The study indicated that the ColV plasmids are heterogeneous. Southern and colony hybridization analyses showed that in most of the colicin V-producing intestinal *E. coli* strains, the colicin V genes are located in the chromosome (14 of 16); in most of the colicin V-producing *E. coli* strains isolated from the blood, they are located in plasmids (18 of 22). In both intestinal and blood *E. coli* isolates, the *traT*, *iss*, and aerobactin receptor genes were present at similar frequencies, but the frequency of the aerobactin synthesis genes was significantly different. The aerobactin receptor gene was present in 25% of the intestinal *E. coli* strains that lack the aerobactin synthesis gene. In the blood isolates, the aerobactin synthesis and receptor genes were present at almost equal frequencies. Among the colicin V-producing isolates, the *iss*, *traT*, and *iu* genes were present in 95.5, 86.4, and 90.9% of the blood isolates and in only 68.8, 43.8, and 81.3% of the intestinal isolates, respectively. The ColV plasmids from blood isolates that were tested for the presence of *traT*, *iss*, and *iu* genes were homogeneous and had DNA sequences that hybridized with each of the probes. On the other hand, the two intestinal strains containing ColV genes in a plasmid were heterogeneous in regard to the carriage of these genes. The presence of ColV is not restricted to specific O types.

ColV plasmids endow *Escherichia coli* with several properties that increase its virulence, including complement and phagocytosis resistance and aerobactin-mediated iron uptake (1, 2, 29). Clinical, epidemiological, and animal studies indicate that *E. coli* harboring the ColV plasmid is more pathogenic and is frequently isolated from extraintestinal infections in animals and humans (3, 4, 7, 19, 23).

The ColV plasmids are heterogeneous in size, genetic composition, ability to conjugate, and expression of virulence properties (12, 14, 16, 18). This suggests that the detection of colicin V production may not necessarily indicate the virulence potential of a given plasmid and the *E. coli* strain which harbors it. Moreover, the effects of various independent virulence-related ColV properties on the ability of ColV to increase virulence are far from clear, as is the potential clinical relevance of such effects (2, 7, 23, 29).

In the current study, the originally described ColV plasmids ColV-CA7 (ColV1), ColV-K94 (ColV2), and ColV-K30 (ColV3) were examined for the presence of the virulence-related factors *traT*, *iss*, and aerobactin production (1, 8, 16, 29). The presence of these properties was also correlated with the presence of ColV plasmids and colicin V production in intestinal and blood isolates of *E. coli*.

MATERIALS AND METHODS

The following *E. coli* strains were used in this study: strains containing the originally described ColV plasmids ColV-CA7 (ColV1), ColV-K94 (ColV2), and ColV-K30 (ColV3) (16); a strain containing a ColV plasmid, ColV-FC201, isolated from a patient with meningitis (2); and 200 strains isolated from the stools of children with diarrhea in Santiago, Chile (100 isolated during the warm season of 1982 to 1983 and 100 isolated during the same period in 1983 to

1984 [11]). The 100 strains isolated in 1982 to 1983 included 17 enterotoxigenic *E. coli* strains, 2 enteropathogenic *E. coli* strains, and one strain that produced Vero toxin. There were no variations in the incidence of diarrhea in the population studied during this period. The study also included 146 *E. coli* strains isolated from patients with bacteremia in the United States and Europe.

Bacteria were grown in Luria-Bertani medium (GIBCO Laboratories, Madison, Wis.). The restriction enzymes were used according to the instructions of the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). ³²P-labeled deoxynucleotides were from Amersham Corp. (Arlington Heights, Ill.). Colicin V production was detected as described elsewhere (25). The intestinal and blood *E. coli* isolates that contained the colicin V genes within a plasmid were serotyped at the Canadian Center for Disease Control, Ottawa, Ontario, by methods previously described (26).

Plasmid DNA was extracted by the method of Birnboim and Doly (9), electrophoresed in 1% agarose slab gels using Tris-borate buffer for 6 h at 120V, transferred onto nitrocellulose by the Southern procedure (24), and hybridized using ³²P-labeled DNA probes. Colony hybridization was done as described elsewhere (22). Probes were prepared by digesting the plasmid DNA with the appropriate restriction enzyme and then separating the fragments by electrophoresis in 1% agarose (SeaKem; FMC Corp., Marine Colloids Div., Rockland, Maine) with Tris-acetate. The DNA probe fragments were cut from the gel, purified as described elsewhere (27), and labeled with α-³²P-deoxynucleotides by nick translation (17). The *traT* probe was a 600-base-pair (bp) *Bst*EII fragment from pKT107 (20); the aerobactin synthesis genes probe was a 1,720-bp *Ava*I fragment from pABN-5 (6); the aerobactin receptor gene probe was a 1,900-bp *Pvu*II fragment from pABN-1 (6); the *iss* gene probe was a 446-bp *Bgl*II-*Tha*I fragment from pH2 (8); and the colicin V probe

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FIG. 1. Hybridization pattern of representative ColV plasmids with virulence-related gene probes. (A) Agarose gel stained with ethidium bromide; (B to E) hybridization patterns of ColV plasmids (lanes: 1, ColV1; 2, ColV2; 3, ColV3; and 4, ColV-FC201) with the *traT* gene probe (B), aerobactin receptor gene probe (C), aerobactin synthesis genes probe (D), and *iss* gene probe (E). Molecular size standards: ColV1, 56.6 megadaltons (MDa); ColV2, 95.0 megadaltons (7, 13).

was a 1,500-bp *Bam*HI-*Eco*RI fragment containing *cvaA* and *cvaB* genes from pHK11 (12). The filters were exposed to X-Omat X-ray film (Eastman Kodak Co., Rochester, N.Y.) for 72 h at -70°C as previously described (17).

RESULTS

Detection of complement and phagocytosis resistance (*traT*, *iss*) and iron uptake DNA sequences in representative ColV plasmids. Previous studies suggested that the originally described ColV plasmids were associated with unique virulence-related properties, although firm experimental evidence was lacking (2, 7, 10, 29). Figure 1 and Table 1 summarize the results of Southern hybridizations, with the *traT*, *iss*, and aerobactin DNA probes (*iuc* and *iut*), on plasmid DNA extracted from *E. coli* strains containing the previously described ColV plasmids CA7 (ColV1), K94 (ColV2), and K30 (ColV3) and a clinical isolate containing the ColV plasmid FC201. The only plasmid to hybridize with each of the probes was ColV-FC201 from *E. coli* isolate FC007. The ColV3 plasmid hybridized with three of the four probes, the *traT* and both aerobactin system gene probes. ColV1 hybridized with the *traT* gene and the aerobactin receptor (*iut*) gene probes, whereas ColV2 hybridized with the *traT* and *iss* gene probes. The *traT* gene probe was the only one able to hybridize with each of the ColV plasmids studied. ColV3 and ColV-FC201 have a complete aerobactin system, ColV1 has the aerobactin receptor gene but not the genes for aerobactin production, and ColV2 lacks both siderophore and receptor genes.

Frequency of ColV-related *traT*, *iss*, and *iut* genes in intestinal and blood isolates of *E. coli*. The above-described

studies with the original ColV plasmids indicate that those plasmids harbor genes for more than one virulence-related trait. To confirm whether this is a general phenomenon and to investigate the relationship between this phenomenon and the ability of ColV⁺ *E. coli* to invade the bloodstream (3, 4, 21), we studied the incidence of the *traT*, *iss*, and *iut* genes in two clinical populations of *E. coli*. With the exception of the aerobactin synthesis genes (*iuc*), there is no marked difference in the frequency of the virulence genes in *E. coli* isolated from the intestine compared with that isolated from blood (Table 2). Blood isolates almost always had a complete aerobactin (*iuc* and *iut*) system; only 2% of the strains had the receptor genes (*iut*) in the absence of the siderophore production genes (*iuc*). In contrast, among the 100 intestinal strains there were 14 strains that had the receptor gene but no aerobactin siderophore synthesis genes. Thus, 25% (14 of 56; Table 2) of the intestinal strains which had the aerobactin receptor gene lacked the aerobactin synthesis genes, as demonstrated by their inability to hybridize with the aerobactin synthesis DNA probe.

If the virulence properties are considered in combination, there are significant differences between extraintestinal and intestinal strains. Of the bacteremic strains, 22% contained the *iss* gene and the *iuc* genes, whereas in the intestinal strains, only 8% contained both genes. According to the

TABLE 2. Frequency of *traT*, *iss*, and *iut* genes in clinical isolates of *E. coli*^a

Source of <i>E. coli</i> isolates	% Frequency of gene			
	<i>traT</i>	<i>iss</i>	<i>iuc</i>	<i>iut</i>
Bacteremia				
Entire population ^b (includes colicin V ⁺)	44	28	62	64
Colicin V ⁺ ^c	86.4	95.5	90.9	90.9
Intestine				
Entire population ^b (includes colicin V ⁺)	41	25	42	56
Colicin V ⁺ ^d	43.8	68.8	81.3	81.3

^a Determined by colony hybridization with the probes described in Materials and Methods.

^b A total of 100 strains were studied.

^c Includes 22 colicin V⁺ strains.

^d Includes 16 colicin V⁺ strains.

TABLE 1. Presence of *traT*, *iss*, and *iut* genes in representative ColV plasmids^a

ColV plasmid	Presence of gene			
	<i>traT</i>	<i>iss</i>	<i>iuc</i>	<i>iut</i>
CA7 (ColV1)	+	—	—	+
K94 (ColV2)	+	+	—	—
K30 (ColV3)	+	—	+	+
FC201	+	+	+	+

^a Determined by Southern hybridization with the probes described in Materials and Methods.

FIG. 2. Hybridization of selected intestinal and blood colicin V-producing *E. coli* strains with colicin V DNA probe. Agarose gel electrophoresis (A) and Southern hybridizations (B) of plasmids isolated from intestinal (DColV) and blood (BColV) *E. coli* strains. Lanes: 1, BColV15; 2, BColV136; 3, BColV118; 4, BColV125; 5, BColV128; 6, ColV-CA7 (control); 7, DColV66; 8, DColV65; 9, DColV120; 10, DColV121; 11, DColV166; 12, DColV169. Molecular size standards: 95 megadaltons, ColV2; 56.6 megadaltons, ColV1; 65 and 42.6 megadaltons, plasmids from *E. coli* TR438 (5, 7, 13).

Fisher exact test ($P = 0.005$), this is a significant difference. Likewise, significant differences were found between the strains when *traT-iuc* (37 versus 18%, $P = 0.003$) and *iss-traT-iuc* (15 versus 3%, $P = 0.003$) were considered.

Location of colicin V genes in intestinal and blood *E. coli* isolates. *E. coli* strains (200 intestinal and 146 bacteremic) were tested for the production of colicin V as previously described (25). Of these, 16 of the intestinal strains and 22 of the blood isolates were found to produce colicin V. Colony and Southern hybridizations with the colicin V gene DNA probe were performed on each colicin V-producing strain in order to identify the ColV plasmid. The probe hybridized with a plasmid in only 2 of 16 colicin V-producing intestinal strains. In the other 14 strains, the probe hybridized with the chromosome, as demonstrated by a positive colony and a negative Southern DNA hybridization. In contrast, 18 of 22 blood isolate strains had the colicin V genes located in a plasmid (Fig. 2). None of the 20 intestinal strains identified as expressing intestinal virulence properties harbored ColV (data not shown).

Frequency of *traT*, *iss*, and *iuc* gene sequences in colicin V-producing *E. coli* from intestines and blood. The colicin V-producing intestinal and bacteremic strains were examined for the presence of the virulence factors *traT*, *iss*, and *iuc* by colony and Southern hybridizations with the appropriate DNA probes. In general, there was a consistently higher percentage of ColV-producing blood isolates that carried the virulence factors than ColV-producing intestinal strains (Table 2). The *traT* gene was found in 86.4% of the bacteremic strains compared with 43.8% of the intestinal strains. Likewise, 95.5% of the bacteremic strains carried the *iss* gene compared with only 68.8% of the intestinal strains. In the

case of the aerobactin system genes (*iuc* and *iut*), the results were the same, although less striking, with 90.9% of the bacteremic strains versus 81.3% of the intestinal strains carrying these genes (Table 2). When blood isolates and intestinal strains that carry the colicin V genes within the chromosome were compared, there was a rather consistent absence of the *traT* gene. Among the colicin V-producing intestinal strains, 9 of 14 (64%) were *traT* negative. Likewise, three of four (75%) of the colicin V-producing blood isolates were *traT* negative.

Of the 18 blood isolates containing ColV plasmids, 11 were chosen for further study. It was found that each of the ColV plasmids hybridized with the *traT*, *iss*, and *iuc* DNA probes. In contrast, the ColV plasmids from the only two intestinal strains which carried the colicin V genes in a plasmid had different hybridization patterns with these probes. The plasmid from intestinal strain D-187 was unable to hybridize with any of the probes, while the strain D-160 plasmid hybridized with the *traT*, *iuc*, and *iut* but not with the *iss* DNA probe (Table 3). These strains also belong to a variety of serotypes (Table 3).

DISCUSSION

The current study confirms the heterogeneity of ColV plasmids found in different strains of *E. coli* and suggests that the detection of colicin V production may not be an adequate way of detecting the virulence potential of these different plasmids and the bacterial strains harboring them. From the data, it can be seen that the originally described ColV plasmids and the intestinal isolates are heterogeneous with regard to the presence of several factors related to

TABLE 3. Presence of *traT*, *iss*, and *iuc* genes in ColV plasmids^a

ColV plasmid ^b	Serotype ^c	Presence of gene			
		<i>traT</i>	<i>iss</i>	<i>iuc</i>	<i>iut</i>
D160	O20:H's rough	+	—	+	+
D187	O?:H10	—	—	—	—
B21	O18:H7	+	+	+	+
B37	OR:K1:H7	+	+	+	+
B40	O?:H4	+	+	+	+
B41	O9:nonmotile	+	+	+	+
B47	O18:K1:H7	+	+	+	+
B48	O18:K1:H33	+	+	+	+
B118	O?:nonmotile	+	+	+	+
B125	O?:H19	+	+	+	+
B128	O2:K1:H4	+	+	+	+
B129	O?:weakly motile	+	+	+	+
B136	O4:nonmotile	+	+	+	+

^a Determined by Southern hybridization with the probes described in Materials and Methods.

^b D, Intestinal strains; B, blood isolates.

^c Determined as described elsewhere (26).

virulence (Tables 1 and 3). In contrast, the ColV plasmids found in blood isolates are homogeneous and contain all the virulence properties examined in this study, confirming previous findings on the relationship between ColV, the presence of virulence-related properties, and extraintestinal infection (4, 21). This relationship is further confirmed by our finding of a lack of ColV plasmids among intestinal strains unable to produce extraintestinal infection, such as those 20 expressing intestinal virulence properties (4, 21). The heterogeneity of the ColV1, ColV2, and ColV3 plasmids also confirms their intestinal origin (16) and is a caution against their use as models for studying the role of ColV properties in extraintestinal infection. It is possible to explain these findings by hypothesizing that the ColV plasmids have arisen from conjugative plasmids that acquired the colicin V genes and the virulence genes by recombination (18) and that the presence of all these genes in one genetic structure imparts a selective advantage to *E. coli*. These findings seem to suggest that even when these ColV-related virulence genes individually can increase *E. coli* virulence, a full complement of them is needed to endow *E. coli* with the ability to invade the bloodstream in humans (Table 3) (1, 2, 3, 7, 29).

An unexpected finding of this study is the large proportion of the intestinal strains that harbor the colicin V genes in the chromosome, unlike the strains isolated from the bloodstream. This integration is probably mediated by the insertion sequences present in the ColV plasmid and in the chromosome (28). These ColV plasmids appear to be most often *traT* negative and nonconjugative. This could be explained by the finding of Darken and Savage, who showed that ColV plasmids repressed for conjugation adhered in higher numbers in an assay with murine small intestine tissue, thereby conferring a selective advantage to nonconjugative ColV plasmids (10). The variety of antigenic composition of *E. coli* harboring the ColV plasmids shows that these organisms are not epidemic clones and suggests that the ColV plasmids are able to transfer fully among different *E. coli* strains, endowing those strains simultaneously with several virulence-related properties.

This study also shows that most *E. coli* isolated from the blood have a complete aerobactin system. Contrary to this, a significant number of intestinal *E. coli* strains have the aerobactin receptor in the absence of aerobactin. This find-

ing supports the contention that the presence of the receptor, in the absence of aerobactin production, is a selective advantage for intestinal strains that are capable of using the aerobactin produced by other bacteria within the intestine (11, 15). When the aerobactin system is present in colicin V-producing strains from blood or the intestine, it is always complete, since the strains that have the receptor gene also have the aerobactin synthesis genes (Table 2). Finally, these results underline the polygenic nature of bacterial virulence, which requires the simultaneous expression of several chromosomal and plasmid-encoded properties.

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