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NOTES

Increased Frequency of ColV Plasmids and Mannose-Resistant Hemagglutinating Activity in an *Escherichia coli* K1 Population

MARIA E. AGÜERO, HARRIETT HARRISON, AND FELIPE C. CABELLO*
Department of Microbiology, New York Medical College, Valhalla, New York 10595

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The expression of traits linked to pathogenicity was studied in a population of *Escherichia coli* K1 strains. It was found that *E. coli* K1 strains isolated from extraintestinal infection harbor the ColV plasmid and express mannose-resistant hemagglutinating activity type VI with a high frequency. The presence of these properties may play a role in the ability of some *E. coli* K1 serogroups to invade.

The K1 capsular antigen of *Escherichia coli* has been implicated in the ability of this bacterium to invade the blood and the meninges of human neonates and the renal tissue of older children (19, 20). Epidemiological studies have demonstrated that *E. coli* K1 strains comprise 77% of the *E. coli* isolated from patients with neonatal meningitis. In addition, 7 to 38% of healthy children may carry *E. coli* K1 in their intestines. The diverse ability of the *E. coli* K1 strains to colonize the neonatal gut, the variation in their pathogenicity for animals and humans, and the fact that only a small percentage of colonized children develop bacteremia and meningitis could be the manifestation of a genetic heterogeneity of these strains with respect to the presence of other pathogenic properties (1, 5, 20). Clinical, epidemiological, and experimental studies have established a correlation between pathogenetic potential and the presence of ColV and R plasmids, the ability to hemolyse and hemagglutinate erythrocytes of different species, and resistance to serum (6, 15). Because the presence or absence of these characteristics could account for the variability of different *E. coli* K1 strains in their ability to colonize and produce disease, we have undertaken the present study to survey virulence-related traits in a population of *E. coli* K1.

The *E. coli* K1 strains were obtained from different sources. A total of 48 strains isolated from the blood, urine, and spinal fluid were examined. Fourteen strains were isolated from the stools of healthy people.

All of the *E. coli* K1 strains received were biochemically characterized as described by Edwards and Ewing (11). Their O, H, and K antigens were characterized by F. and I. Ørskov, Staten Serum Institute, Copenhagen, Denmark. The antibiotic susceptibility of the *E. coli* K1 isolates was determined by the Bauer-Kirby method (4). The presence of K1 antigen was ascertained with group B meningococcus antiserum and with K1-specific bacteriophages (5, 14). Hemolytic ability was detected in Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep blood. Hemagglutination of group A human, bovine, and African green monkey erythrocytes was studied with bacteria grown in colonization factor antigen agar, in the presence and absence of 1% mannose as described previously (12). The colicin production of all *E. coli* K1 isolates was tested by the agar overlay method (25). Strains were considered producers of colicin if they inhibited the growth of the *E. coli* K12 C600 strain. Spots of the *E. coli* K1 strains were also overlaid with soft agar containing a C600 strain carrying a ColV plasmid conjugated from one *E. coli* K1 strain. This plasmid was classified as ColV because its presence endows *E. coli* K1 C600 with immunity to ColV standard strains. Colicin producer strains that did not inhibit the growth of this C600 ColV$^+$ strain were considered producers of colicin V. The presence of two or more antibiotic resistance markers and the ability to transfer antibiotic resistance markers by conjugation were taken as indications of the presence of R plasmids. Conjugation experiments were performed as described by Anderson (3), using an *E. coli* K12 C600 *hsr*, *hsm*, nalidixic acid-resistant strain as a recipient. The Fisher test was used to analyze the incidence of the different pathogenic traits in the *E. coli* K1 population (21).

The group of *E. coli* K1 strains isolated from

TABLE 1. Incidence of ColV, hemagglutinating, and hemolysin activities and R plasmids among *E. coli* K1 strains from different sources[a]

Strain source	No. of strains	ColV	No. of strains positive for		
			MRHA[b]	Hemolysin	R plasmids
Extra intestinal					
Blood	32	17 (53)	17 (53)	3 (9.3)	19 (58)
CSF	12	3 (25)	9 (75)	4 (33)	2 (16)
Urine	4	2 (50)	2 (50)	2 (50)	2 (50)
Mean		(46)	(58)	(19)	(48)
Intestinal	14	3 (21)	1 (7)	6 (43)	6 (43)
Mean		(40)	(47)	(24)	(47)

[a] Numbers in parentheses are percentages.
[b] MRHA, Mannose-resistant hemagglutination for group A human erythrocytes.

extraintestinal infection had a high incidence of production of colicin V when compared with the strains isolated from stools of healthy individuals (Table 1). Extraintestinal *E. coli* K1 produced colicin V more frequently than did intestinal *E. coli* K1, 42 over 21% ($P = 0.074$). Among the group of extraintestinal strains, the *E. coli* K1 strains isolated from blood produced colicin V with higher frequency than did those of any other group, especially when compared with intestinal *E. coli* K1, 51 versus 21% ($P = 0.043$). In the extraintestinal group of strains, the differences in colicin V expression between strains isolated from blood and the spinal fluid were noteworthy ($P < 0.081$). It should be mentioned that the incidence of ColV among these strains may be even larger because ColV may be lost upon storage (9). Furthermore, since there are several immunity groups among the ColV plasmids, we may be underestimating its presence by detecting immunity with only one ColV plasmid (10). A total of 58% of the strains isolated from extraintestinal infection had mannose-resistant hemagglutinating activity, compared with 7% of the intestinal group ($P < 0.001$; Table 1); 86% of these strains also hemagglutinated green monkey erythrocytes but not bovine erythrocytes, indicating that they belong to the hemagglutination type VI (13). It is noteworthy that 75% of the strains isolated from the spinal fluid expressed mannose-resistant hemagglutination (MRHA) and that they harbored ColV and R plasmids with low frequency, indicating, perhaps, the singularity of this group of strains. There is a significant difference between the extraintestinal and intestinal isolates regarding the expression of hemolytic activity ($P < 0.020$); there is not a significant difference between them regarding their carriage of R plasmids (Table 1). The analysis of the strains according to their O antigen indicates that 62% belong to the serogroups O1, O18, ab, ac, and untypable. There were no significant differences between these serogroups of strains and the rest when they were analyzed for the presence of ColV, MRHA, hemolytic activity, and R plasmids. It is significant that all of the strains of serogroup O1 are not hemolytic and do not harbor R plasmids. The analysis of these common serogroups according to their origin indicates that they can be found almost equally distributed among intestinal or extraintestinal isolates and that among the extraintestinal strains, the most common serogroups are equally distributed among blood, cerebrospinal fluid, and urine isolates (data not shown). The biochemical tests of this group of *E. coli* K1 strains did not detect other plasmids or chromosomally mediated properties that have been related to increased virulence, i.e., dulcitol fermentation or urea production (16).

Our results show that the ColV plasmid is present in a high percentage of these *E. coli* K1 strains, especially in those isolated from blood. The high incidence of ColV plasmids among these strains could be related to the pathogenic potential of these bacteria. For example, the increase in pathogenicity produced by the presence of ColV in the *E. coli* K1 strains could be due to an increase in serum resistance and resistance to phagocytosis mediated by outer membrane proteins specified by the ColV plasmid (2). Our preliminary results also indicate that *E. coli* K1 strains can harbor ColV plasmids specifying an iron uptake system which may occur alone or in conjunction with functions that augment resistance to serum (26). The ability to compete for iron may be crucial for invasion of the bloodstream and colonization of the intestine of the breast-fed neonate, because in those places, the concentration of iron available for microbial growth is low (18). The ability to colonize the intestines of neonates could also be increased by the ColV-specified genes that mediate adherence of ColV-harboring bacteria to mouse intestinal epithelium (8).

The high frequency of display of mannose-resistant hemagglutination type VI in the *E. coli* K1 population isolated from extraintestinal infection is expected for this type of population (13). This property has been linked to surface-

associated antigens involved in bacterial adhesion to eucaryotic cells and in the colonization of the host by *E. coli* (24). Although the role that this property plays in neonatal K1 disease is unknown, it could increase the ability of bacteria to colonize the intestinal tract, enhancing opportunities to invade.

The ability to lyse erythrocytes was found more frequently among intestinal than in extraintestinal *E. coli* strains; this could be a reflection of the fact that, as communicated by others, few *E. coli* K1 strains are able to express both hemagglutination type VI and hemolysis (13). The explanation for these facts is not known.

To our knowledge, this is the first report indicating that *E. coli* K1 strains isolated from human disease have a higher incidence of ColV and MRHA than do other *E. coli* populations. Minshew et al. (15) and Silver et al. (22) found that a very small percentage of *E. coli* strains harbor ColV plasmids and that the plasmid was absent from all *E. coli* organisms isolated from the normal flora. Their findings contrast with ours and those of Davies et al. (10), which show that approximately 25 to 30% of *E. coli* organisms isolated from extraintestinal infection have the ColV plasmid and that this plasmid is present in a relatively important number even among intestinal isolates. These differences could be due to methodological differences in screening for ColV presence or to epidemiologically different *E. coli* populations.

Regarding the distribution of serogroups, our relatively small strain population is representative and comparable to other *E. coli* K1 populations (1, 7, 22), perhaps validating the general relevance of our findings for *E. coli* K1. The reasons for association of a few O antigens with K1 antigen, MRHA, and ColV are not known. It could be assumed that a few clones of *E. coli* K1 strains are better adapted to produce disease and that their association with ColV plasmids or MRHA increases their invasiveness (1, 17). Conversely, there may be genetic linkage between the genes coding for these properties (O and K1 antigens, MRHA) in the chromosome, and these genes or closely linked genes may confer upon *E. coli* strains properties that allow them to receive and maintain plasmids, such as ColV. These results, our experimental data (2), and data from other laboratories (23) support the idea that the pathogenicity of *E. coli* K1 strains is a multifactorial phenomenon in which chromosomal and plasmid gene products may interact to increase the pathogenic potential of this group of *E. coli* strains. Prospective studies with increased numbers of clinical isolates and more experimental work will be needed to discern the relevance of ColV and MRHA in *E. coli* K1 human disease and to fully understand some of our findings.

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