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Characterization of *Escherichia coli* Serotype O157:H7

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A total of 174 strains of *Escherichia coli* serotype O157:H7 representing human isolates obtained from outbreaks and sporadic cases of hemorrhagic colitis, hemolytic-uremic syndrome, and nonbloody diarrheal illnesses as well as from asymptomatic carriers across Canada and the United States were examined. *E. coli* serotype O157:H7 possessed distinct biochemical markers, a 100% negative reaction for β-glucuronidase and sorbitol, and a 100% positive reaction for raffinose and dulcitol; all strains otherwise were biochemically typical of *E. coli*. The vast majority (97%) of the strains were susceptible to commonly used antimicrobial agents. All strains produced readily detectable levels of Verotoxin; however, with polymyxin extraction, nearly 50% of the strains showed up to a 10-fold increase in the toxin level. None were found to mediate hemagglutination of human group A erythrocytes with or without D-mannose. The majority (~70%) of the strains showed localized and diffuse adherence to HEp-2 cells and Henle 407 cells, and the adherence patterns were not very different from those observed among other *E. coli* strains. Twenty phage types were recognized, with phage types 1 and 2 accounting for 65% of the test strains. Plasmid analysis indicated three basic plasmid profiles: profile I was characterized by 68.7- and 4.2-megadalton (MDa) plasmids (62% of strains), profile II was characterized by 66.2- and 1.8-MDa plasmids (20% of strains), and profile III was characterized by a 62.5-MDa plasmid (18% of strains). A small number (19%) of the strains carried at least one additional plasmid over the basic complements, and these could be considered to constitute a miscellaneous category. None of the above-described characteristics of *E. coli* serotype O157:H7 could be directly correlated with one another, with the nature of infection, or with the geographical distribution of strains.

Escherichia coli serotype O157:H7, initially recognized in 1982 in the United States (34, 42), has now emerged as an important enteric pathogen of considerable public health significance in Canada, the United States, and the United Kingdom, with many outbreaks and numerous sporadic cases of hemorrhagic colitis, hemolytic-uremic syndrome, and diarrheal illness occurring in nursing homes, day-care centers, schools, and the community (5, 6, 16, 20, 22, 29, 31, 33, 35, 39, 40; International Symposium and Workshop on Verocytotoxin-producing *Escherichia coli* Infections, Toronto, Ontario, Canada, 12 to 15 July 1987). *E. coli* serotype O157:H7 has been isolated from cattle and foods of animal origin (4, 10); person-to-person transmission is also an important mode of spread of this agent (6, 32, 40). Hemorrhagic colitis and hemolytic-uremic syndrome arise as complications of infection with *E. coli* serotype O157:H7, and these may be associated with high morbidity and mortality (6, 35, 39). In view of the importance of *E. coli* serotype O157:H7 in human diseases, we carried out a study to characterize this agent. A total of 174 strains of *E. coli* serotype O157:H7 representing human isolates obtained from outbreaks and sporadic cases of hemorrhagic colitis, hemolytic-uremic syndrome, and nonbloody diarrheal illnesses as well as from asymptomatic carriers across Canada and the United States were examined for biochemical properties, antimicrobial susceptibility patterns, hemagglutination and adherence properties, and phage type and plasmid profiles.

(Results of this study were presented at the International Symposium and Workshop on Verocytotoxin-Producing *Escherichia coli* Infections, Toronto, Ontario, Canada, 12 to 15 July 1987 [hereafter referred to as International Symposium] [S. Ratnam, S. B. March, R. Ahmed, G. S. Bezanson, T. R. Patel, and S. Kasatiya, abstr. no. CEP-11].)

MATERIALS AND METHODS

***E. coli* serotype O157:H7.** All test strains were human isolates either isolated in the Newfoundland and Labrador Public Health Laboratories, St. John's, Newfoundland, Canada (20 strains), or obtained from the following sources in Canada and the United States: Provincial Public Health Laboratory in Halifax, Nova Scotia (8 strains), Fredericton, New Brunswick (9 strains), Sainte-Anne-de-Bellevue, Quebec (27 strains), Toronto and Ottawa, Ontario (38 strains), Regina, Saskatchewan (4 strains), and Vancouver, British Columbia (17 strains); Hospital for Sick Children, Toronto, Ontario (2 strains); Foothill's Hospital, Calgary, Alberta (21 strains); Children's Hospital, Vancouver, British Columbia (6 strains); Laboratory Centre for Disease Control, Ottawa, Ontario (4 strains); Washington State Public Health Laboratory, Seattle (3 strains); University of Washington School of Medicine, Seattle (6 strains); and Centers for Disease Control, Atlanta, Ga. (9 strains). These isolates represented at least 80 cases of bloody diarrhea, 21 cases of nonbloody diarrhea, and 15 cases of hemolytic-uremic syndrome and 10 asymptomatic persons. Case histories were not readily available for the remaining isolates. The U.S. strains included the Oregon and Michigan outbreak isolates (34) as well as isolates from sporadic cases of bloody diarrhea.

Laboratory procedures. Test strains were confirmed as *E. coli* by standard biochemical tests, serotyped with O157 and H7 antisera (Laboratory Centre for Disease Control) by

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standard methods (12), and maintained on brain heart infusion and blood agar media until tested.

Biochemical properties were determined by standard methods (12) and by the API 20E and API ZYM systems (Analytab Products, Plainview, N.Y.). β-Glucuronidase was tested by the 4-methylumbelliferyl-β-D-glucuronide test in accordance with the manufacturer's instructions (Difco Laboratories, Detroit, Mich.). Antimicrobial susceptibility patterns were determined by the standard disk diffusion test (2). The Verotoxin assay was carried out with or without polymyxin extraction of the cultures as described by Karmali et al. (19, 20). The hemagglutination test was done as previously described (11, 38). Briefly, cultures were propagated on colonization factor antigen medium (11), and overnight cultures were suspended in 0.85% NaCl (10^{10} CFU/ml) and tested in equal volumes with a suspension of human group A erythrocytes with or without 1% D-mannose. Three strains of *E. coli* (E6237B, colonization factor antigen I; E1392, colonization factor antigen II; and E11881A; all from B. Rowe, Central Public Health Laboratory, London, United Kingdom) served as controls for the hemagglutination test. The cell culture adherence test was carried out with HEp-2 (Flow Laboratories, Inc., Mississauga, Ontario, Canada) and Henle 407 (American Type Culture Collection, Rockville, Md.) continuous cell lines by a previously described method (27). Two strains of *E. coli*, JCP-88 and ATCC 25922 (27), served as standard controls for the adherence test along with many adherent enteropathogenic *E. coli* strains belonging to serogroups O26, O55, O111, and O119 (36). Phage typing was done by the method of Ahmed et al. (1). Plasmid profile analysis was carried out with unaerated, overnight tryptone soya broth cultures (Oxoid, Ottawa, Ontario, Canada) by the modified alkali-detergent method described by Dillon et al. (8). Restriction endonuclease digests were carried out in buffers and under conditions specified by the manufacturer (Boehringer Mannheim, Montreal, Quebec, Canada). Electrophoresis was conducted at room temperature in Tris-acetate buffer (pH 7.8) with vertical 0.75% agarose gels.

Analysis. In addition to their application for the overall characterization of *E. coli* serotype O157:H7, the data generated were also analyzed in an attempt to determine correlations, if any, between the various test parameters used to characterize the agent and in relation to the geographic distribution of the strains and the nature of the infections, i.e., hemorrhagic colitis, hemolytic-uremic syndrome, nonbloody diarrhea, asymptomatic infection, etc., associated with individual strains.

RESULTS

The biochemical reactions of *E. coli* serotype O157:H7 are summarized and compared with those of other *E. coli* in Table 1. *E. coli* serotype O157:H7 possessed biochemical markers that were significantly different from those of other *E. coli*. These included a 100% negative reaction for β-glucuronidase and sorbitol and a 100% positive reaction for raffinose and dulcitol. Sucrose was fermented by 87% of *E. coli* serotype O157:H7 strains, in contrast to a range of 42 to 54% for strains of other *E. coli*. The great majority (87%) of test strains fermented dulcitol and sucrose within 24 h, while a slightly larger percentage (89%) of the strains failed to ferment rhamnose within this period. Thus, upon initial testing, a biotyping scheme based on combinations of dulcitol, sucrose, or rhamnose fermentation reactions after a 24-h incubation period appeared feasible. However, in subsequent tests with up to 10 colonies of the test strains, these reactions were found to be inconsistent and variable within individual strains. Owing to the inconsistencies, some outbreak strains otherwise having identical phage type and plasmid profiles turned out to have multiple and different biotype profiles when retested on different occasions. The API 20E profiles of the test strains were slightly different from those observed for other *E. coli* strains because of the variation in sorbitol fermentation. A major profile (85% of strains) and some minor profiles of *E. coli* serotype O157:H7 were observed with this system, the differences for the minor profiles being the variable rhamnose and sucrose reactions, as mentioned before. The indole, citrate, and urease reactions of all test strains were also typical of *E. coli* as determined by API 20E, i.e., indole positive and citrate and urease negative. The API ZYM profile of *E. coli* serotype O157:H7 was determined and compared with those of 50 non-*E. coli* serotype O157:H7 clinical isolates. All strains of both groups of *E. coli* yielded moderately to highly (20 to ≥40 nmol) positive reactions for alkaline phosphatase, leucine aminopeptidase, and acid phosphatase and variably low to moderately (5 to 20 nmol) positive reactions for valine aminopeptidase, trypsin, phosphohydrolase, and β-galactosidase. The rest of the reactions were uniformly negative for both groups. All *E. coli* serotype O157:H7 strains were found to be negative for β-glucuronidase by the API ZYM system as well; however, a large percentage of non-*E. coli* serotype O157:H7 strains were also found to be negative for this enzyme by this system (data not shown).

TABLE 1. Summary of the biochemical reactions of *E. coli* serotype O157:H7 and *E. coli* other than serotype O157:H7[a]

Test	% Positive		
	E. coli serotype O157:H7 (n = 174)	*E. coli* other than serotype O157:H7	
		n = 9,714[b]	n = 1,887[c]
β-Glucuronidase	0	96	NT[d]
Sorbitol	0	95	80
Salicin	0	0	36
Esculin	0	NT	31
Arginine dihydrolase	0	NT	16
Adonitol	0	3	1
Inositol	0	1	1
Cellobiose	0	0	1
Urease	0	0	0
Citrate	0	0	0
KCN	0	NT	0
Sucrose	87	42	54
Glucose (acid)	100	100	100
Glucose (gas)	98	NT	92
Indole	100	99	96
Arabinose	100	99	99
Trehalose	100	99	98
Mannitol	100	99	98
Lactose	100	88	92
Maltose	100	98	91
Rhamnose	100	91	84
Xylose	100	97	83
Lysine decarboxylase	100	92	81
Ornithine decarboxylase	100	74	58
Raffinose	100	20	49
Dulcitol	100	50	49

[a] Based on 48 h of incubation.
[b] Data are taken from reference 21.
[c] Data are taken from reference 12.
[d] NT, Not tested.

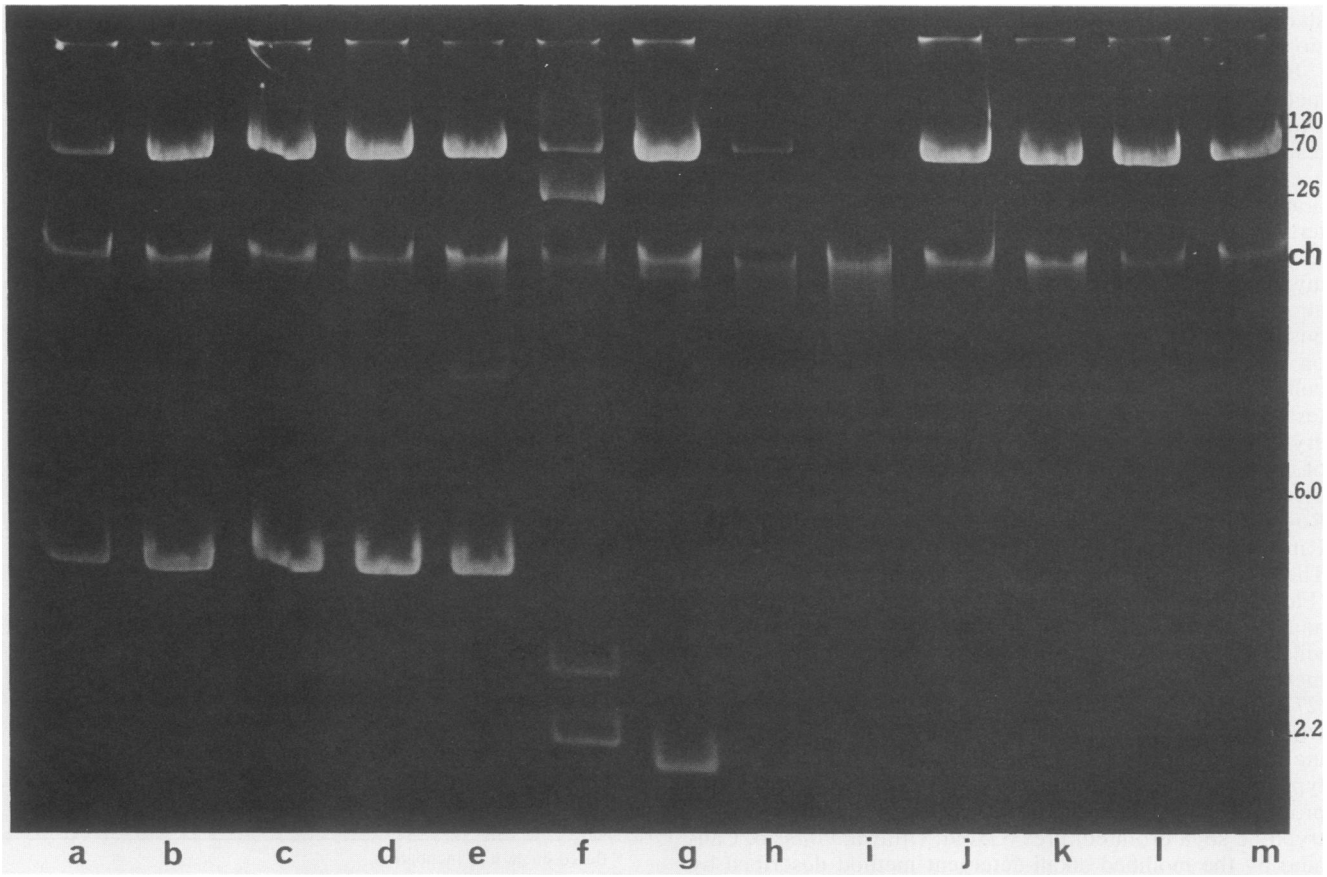

FIG. 1. Major plasmid patterns detected in *E. coli* serotype O157:H7. Lanes: a to e, profile I, 68.7- and 4.2-MDa plasmids; f, marker plasmids (sizes in megadaltons indicated on the right); g to i, profile II, 66.2- and 1.8-MDa plasmids; j to m, profile III, 62.5-MDa plasmid. Electrophoresis was done for 3 h at 80 V. ch, Chromosome. Note that the strain in lane i has lost the 66.2-MDa plasmid.

The vast majority (97%) of the test strains were found to be susceptible to commonly used antimicrobial agents, including ampicillin, cephalothin, carbenicillin, tetracycline, kanamycin, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, sulfisoxazole, nalidixic acid, and nitrofurantoin. Of the 174 strains tested, 1 was found to be resistant to ampicillin, carbenicillin, sulfisoxazole, and tetracycline; 4 others were found to be resistant to sulfisoxazole and tetracycline.

All test strains produced Verotoxin, which was detectable in the cell culture system within 1 to 3 days; in every instance, the toxin was detectable in culture filtrates without polymyxin extraction (19). However, with polymyxin extraction, nearly 50% of the strains tested showed up to a 10-fold increase in the toxin level.

None of the test strains was found to mediate hemagglutination of human group A erythrocytes in either the absence or the presence of D-mannose. However, the majority (~70%) of the strains tested were found to be adherent to the larynx-derived HEp-2 cells and the gut-derived Henle 407 cells; occasionally the adherence was better to one or the other cell line. The strains found to be adherent exhibited either localized or diffuse patterns or both, similar to those previously described for other *E. coli* serotypes (7, 37). However, there were variations in the degree of adherence from light to heavy, i.e., scattered single rods and small clusters adherent to 50% of cells and heavy confluent adherence to ≥90% of cells. There were also inconsistencies in adherence in that the test results were not always 100% reproducible with some strains. There were otherwise no significant differences in the outcomes of adherence tests between cultures grown with or without 1% D-mannose.

Phage typing revealed 20 different phage types among the 174 strains tested. Type 1 was by far the most common (43%), followed by type 2 (22%). Strains from eight of nine outbreaks of hemorrhagic colitis, hemolytic-uremic syndrome, nonbloody diarrhea, and asymptomatic infections belonged to phage types 1 to 5 (1) (see Table 2).

Plasmid analysis revealed three basic plasmid profiles among 100 *E. coli* serotype O157:H7 strains screened: profile I was characterized by plasmids of 68.7 and 4.2 megadaltons (MDa), profile II was characterized by 66.2- and 1.8-MDa plasmids, and profile III was characterized by a 62.5-MDa plasmid (Fig. 1). Extensive testing (8) confirmed that the faint band migrating between chromosomal DNA and the 6-MDa marker in profile I strains (lanes a to e) represented the open circular form of the 4.2-MDa plasmid. Interestingly, the 66.2-MDa plasmid detected in profile II isolates occasionally was observed to be unstable under the laboratory conditions used. Thus, no plasmid of this size could be detected in the strain shown in Fig. 1, lane i, even upon a 10-fold increase in the amount of cells used for DNA recovery.

Based on the three basic profiles, the test strains could be divided into three corresponding groups with membership distributed as follows: group I, 62%; group II, 20%; and group III, 18%. A small number (19%) of strains were found to carry at least one additional plasmid over the basic

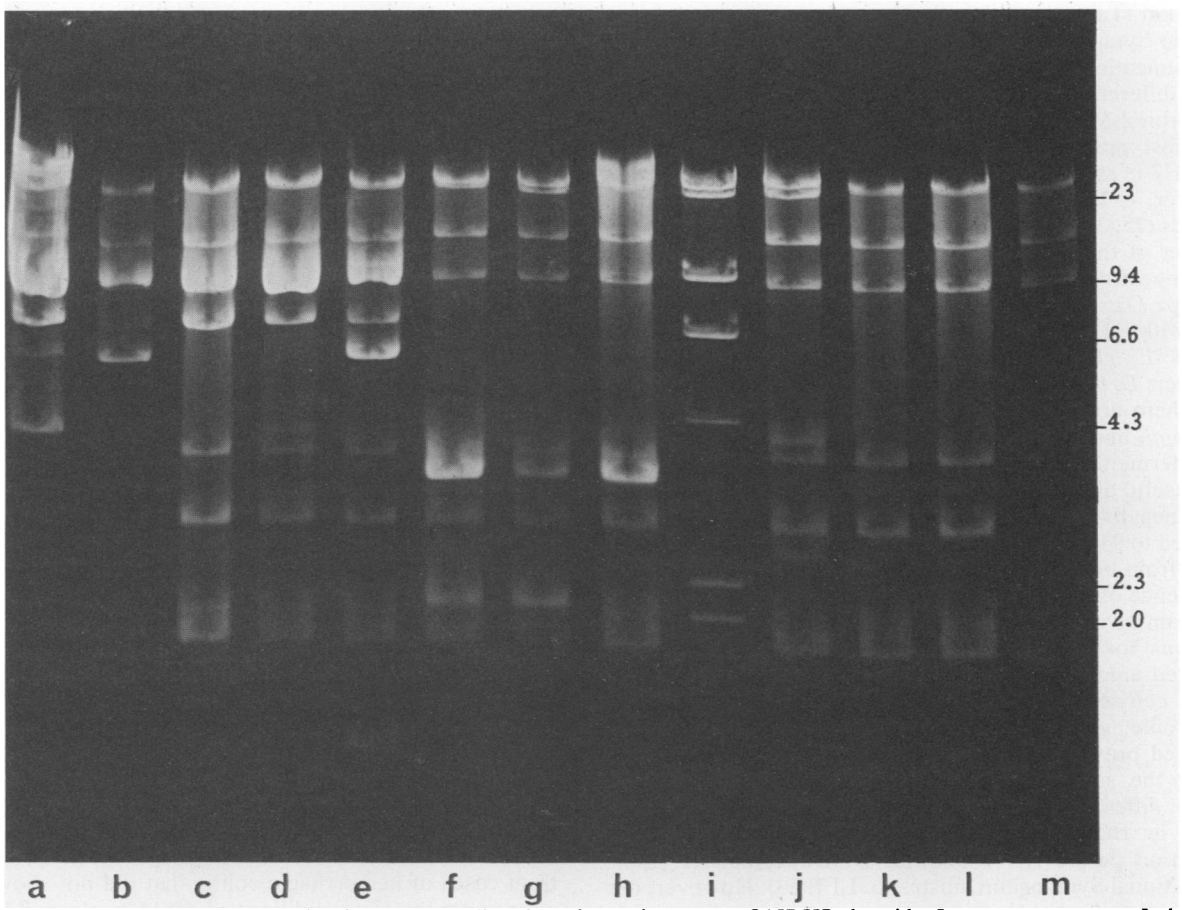

FIG. 2. Fragmentation patterns resulting from *Bam*HI digestion of *E. coli* serotype O157:H7 plasmids. Lanes: a to e, group I plasmids; f to h, group II plasmids; i, lambda DNA digested with *Hin*dIII; j to m, group III plasmids. Electrophoresis was done for 16 h at 24 V. Numbers refer to lambda fragments in kilobases.

complements of groups I, II, and III. This miscellaneous category included as many as 10 distinct plasmid constellations. Digestion of the plasmid preparations with the restriction endonucleases *Bam*HI, *Hin*dIII, and *Eco*RI permitted further differentiation of each of the three basic plasmid groups into at least three subgroups, some of which are illustrated in Fig. 2.

A comparison of the phage type and plasmid profiles of *E. coli* serotype O157:H7 isolates from five outbreaks of hemorrhagic colitis indicated a 100% consistency between the two parameters. In at least two instances, strains displaying the same plasmid profile were of different phage types (Table 2). Throughout the study, in terms of various test parameters, there were no direct correlations or specific patterns among strains in relation to either the nature of infection or their geographical distribution.

DISCUSSION

E. coli serotype O157:H7 is different from other *E. coli* not only from certain clinical and epidemiological standpoints (22) but also in some bacteriological features. A combination of β-glucuronidase, sorbitol, raffinose, and dulcitol reactions can virtually separate this serotype from the rest, and in this respect, our results fully substantiate the observations of Krishnan et al. (21) made with a limited number of *E. coli* serotype O157:H7 strains. *E. coli* has been reported to be the only lactose-fermenting gram-negative bacillus capable of producing β-glucuronidase, and about 96% of all *E. coli* strains produce the enzyme (14). *E. coli* serotype O157:H7 certainly is an exception (Table 1) (9, 21). Of over 1,000 strains of *E. coli* serotype O157:H7 tested to date, none has been found to ferment sorbitol within 24 h, with a single

TABLE 2. Consistency of phage type and plasmid profiles of *E. coli* serotype O157:H7 isolated during outbreaks

Outbreaks		No. of strains tested	Phage		Plasmid		% Correlation
Location	Yr		Type	No. of strains	Group	No. of strains	
Oregon and Michigan	1982	3	3	3	II	3	100
Ottawa, Ontario, Canada	1984	2	5	2	II	2	100
London, Ontario, Canada	1985	5	2	5	III	5	100
Walla Walla, Wash.	1986	3	25	3	II	3	100
St. John's, Newfoundland, Canada	1986	3	4	3	III	3	100

exception (Table 1) (3, 13; A. Borczyk and H. Lior, International Symposium, abstr. no. CEP-9; J. Jessop, personal communication). From this point of view, the use of sorbitol-based differential media such as H7 antiserum-sorbitol (13) and sorbitol-MacConkey (25) has the merit of being a reliable and most practical method of detecting *E. coli* serotype O157:H7 in stools (21, 25). The reliability of this approach, however, is largely dependent on early collection of stool samples (25, 29, 33, 42). *E. hermannii*, a recently recognized member of the genus *Escherichia*, is not only β-glucuronidase negative and a non-sorbitol fermenter like *E. coli* serotype O157:H7 (12) but also has been known to cross-react with O157 antiserum and thus can easily be misidentified as *E. coli* serotype O157:H7 (4a). Unlike *E. coli*, however, *E. hermannii* produces a yellow pigment; in addition, there are certain biochemical differences between *E. hermannii* and *E. coli* (12). Our results confirm that cellobiose fermentation and growth on KCN medium are the most useful differential biochemical tests, both of which are 100% negative in *E. coli* serotype O157:H7 (Table 1) as opposed to 94 to 97% positive in *E. hermannii* (12). A recent report from the Centers for Disease Control (3) indicated the occurrence of biochemically atypical *E. coli* serotype O157:H7 strains with a negative reaction for indole and positive reactions for citrate and urease. Although this might be expected among *E. coli* strains (12), we did not encounter any *E. coli* serotype O157:H7 strains with such a biochemical profile, and this appears to be a rare occurrence, as indicated previously (3). *E. coli* serotype O157:H7 strains having the same phage type or plasmid profiles can be further differentiated by biotyping based on dulcitol, sucrose, or rhamnose fermentation reactions after a 24-h incubation period (R. Khakhria, D. Duck, and H. Lior, International Symposium, abstr. no. LFE-13). However, our study indicated that these reactions are not consistent features of all strains. Therefore, we think that biotyping of *E. coli* serotype O157:H7 strains based on these reactions may be of questionable value or limited use, depending on the strains tested. The biotype profile which is likely to be more consistent is that of positive dulcitol and sucrose reactions and a negative rhamnose reaction. However, this biotype profile accounts for 80% of *E. coli* serotype O157:H7 strains (data not shown) and is therefore, by itself, poorly discriminatory. A biotyping scheme similar to that described above but based on a limited number of *E. coli* serotype O157:H7 strains was recently reported to yield variable results by other workers as well (21).

The antimicrobial susceptibility results obtained with *E. coli* serotype O157:H7 strains collected from wide geographical areas clearly show that significant drug resistance has yet to be encountered among *E. coli* serotype O157:H7 strains, and this observation is consistent with previous reports from other geographical areas (3, 21, 39). Similarly, we also noticed tetracycline resistance in a few strains.

E. coli serotype O157 strains produce at least two distinct cytotoxins referred to as Verotoxin I and Verotoxin II or Shiga-like toxins I and II (22, 39). *E. coli* serotype O157:H7 strains have been shown to produce elevated (moderate or high) levels of the cytotoxins (26). We did not quantitatively determine the levels of toxins produced by individual strains because all strains were found to produce toxins in readily detectable levels, nor did we determine the antigenic types of the toxins detected. However, since it has been subsequently observed that only Verotoxin I (and not Verotoxin II) levels are increased by polymyxin B extraction (G. S. Bezanson; unpublished observation), the percentage (~50%) of isolates displaying a polymyxin effect could be taken as a minimal estimate of the frequency of Verotoxin I producers. There are a number of *E. coli* serogroups other than O157 now known to produce Verotoxin (20, 26; S. M. Scotland, G. A. Willshaw, H. R. Smith, T. Cheasty, and B. Rowe, International Symposium, abstr. no. LFE-1), and *E. coli* serotype O157:H7 does not appear to be unique in this respect.

E. coli serotype O157:H7 has been shown to adhere to surface epithelial cells in animal intestine models (15, 30). In vitro studies done with a few strains of *E. coli* serotype O157:H7 have indicated a characteristic adherence to Henle 407 gut cells but not to HEp-2 cells (18) and a variable degree of both localized and diffuse adherence to both Henle 407 and HEp-2 cells (38). While our results, based on a large number of strains, appear to be more in agreement with those of the latter study, they indicate that the adherence property, as determined by in vitro tests, is, to some extent, a variable feature among *E. coli* serotype O157:H7 strains. This observation is unlike the previous observation with other *E. coli* strains that, within each serotype, the adherence pattern is highly consistent (36). With *E. coli* serotype O157:H7, the adherence property appears to be dependent on the strains and, to some extent, the cell lines used in in vitro adherence tests. In addition, the method used may also influence the test results and interpretation (7, 38; A. D. Junkins and M. P. Doyle, International Symposium, abstr. no. AMV-5). Cell culture adherence studies carried out with other *E. coli* strains have suggested localized adherence to be a virulence factor (23, 36). We found no direct correlation between the adherence patterns of the isolates and the nature of the infections. In fact, there were a few isolates from cases of hemorrhagic colitis that did not show adherence under the test conditions. A 60-MDa plasmid has been reported to code for the adherence property of *E. coli* serotype O157:H7 (18). Since adherence is one of the unstable factors which are easily affected during storage (28), it may be that this plasmid was lost in some of our test strains. In fact, we noted that the 66.2-MDa plasmid present in group II strains was unstable but did not verify its role in adherence. Although the microscopic method we used to determine adherence is not as precise as the culture quantitation method recently used by others for *E. coli* serotype O157:H7 (38), it is the same as the one used for other *E. coli* serotypes (7, 27, 36, 37). Based on our observations, therefore, it can be concluded that the adherence property and patterns of *E. coli* serotype O157:H7 as determined in the cell culture adherence test do not appear to be very different from those observed for other *E. coli* serotypes (7, 24, 27, 36, 37).

We used only human group A erythrocytes to determine the hemagglutination property of *E. coli* serotype O157:H7. Our results are in agreement with earlier findings by other workers who utilized a variety of cells for this purpose (38) and appear to support the view that the adherence property of *E. coli* serotype O157:H7 is mediated by surface adhesins other than nonhemaggutinating pili (18, 38).

A phage typing scheme has been successfully developed for *E. coli* serotype O157:H7 (1), and with its application, over 40 phage types have been recognized so far (H. Lior, personal communication). The present study and those of others clearly indicate that the great majority of *E. coli* serotype O157:H7 strains belong to phage types 1, 2, 4, and 8 (1; Khakhria et al., International Symposium, abstr. no. LFE-13). The phage typing scheme for *E. coli* serotype O157:H7 has proven to be highly discriminatory and has

been reliably used in epidemiological investigations of *E. coli* serotype O157:H7 outbreaks (1, 6).

All 100 isolates of *E. coli* serotype O157:H7 screened carried a large plasmid that ranged from 62.5 to 68.7 MDa. Such variation in the size of the large plasmid of this organism has been observed by others as well (17, 24, 42). Karch et al. (18) reported that a plasmid of this general size ("60 Mda plasmid") plays a key role in the attachment of *E. coli* serotype O157:H7 to epithelial cells. Tzipori et al. (41), on the other hand, recently claimed that the 60-MDa plasmid is not involved in the virulence of *E. coli* serotype O157:H7 in gnotobiotic piglets. At any rate, Levine et al. (24) have taken advantage of the apparent universal occurrence of the 60-MDa plasmid to construct a DNA probe specific for *E. coli* serotype O157:H7. We (Fig. 1, lane i) and others (18) have noted the spontaneous loss of the 60-MDa plasmid during successive culturing of certain *E. coli* serotype O157:H7 strains. Should this loss occur in vivo, probes developed with fragments recovered from this plasmid would be unreliable for routine application. Nevertheless, our study indicated the plasmid profile analysis to be another reliable epidemiologic tool, and we conclude that this analysis could serve as a useful adjunct to phage typing.

The spectrum of clinical manifestations varies widely with *E. coli* serotype O157:H7 infections (29, 35, 40). Our study indicated that none of the characteristics of *E. coli* serotype O157:H7 determined by the parameters we used in characterizing this agent could be directly correlated with the nature of the infections.

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