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Plasmid-Encoded Expression of Lipopolysaccharide O-Antigenic Polysaccharide in Enteropathogenic *Escherichia coli*

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The role of a plasmid in the virulence activity of an enteropathogenic *Escherichia coli* (EPEC) strain belonging to serotype O111:NM was examined. EPEC strain B171, which is resistant to chloramphenicol, streptomycin, sulfathiazole, and tetracycline, harbors a 54-megadalton plasmid, pYR111, and exhibits localized adherence (LA) with HeLa cells. Curing the plasmid yielded strain B171-4, which had lost the ability to exhibit LA, resistance to the antibiotics, and the lipopolysaccharide (LPS) O-antigenic polysaccharide. To confirm that these phenotypic characteristics were specified by pYR111, the plasmid was transferred by conjugation into a nalidixic acid-resistant strain of *E. coli* HB101. LA and antimicrobial resistance were expressed in most of the transconjugants examined. The O-polysaccharide side chains, antigenically reactive with O111-specific antiserum, were also expressed by the transconjugants. Although EPEC plasmids coding for both drug resistance and LA have been described, an EPEC plasmid encoding the expression of an LPS O antigen has not been previously reported. Similar findings described for some *Shigella* and *Salmonella* strains suggest that plasmid-encoded modification of the LPS in some enteric bacterial species may be more common than previously recognized and may contribute to the characteristic virulence activity of the organism.

Enteropathogenic *Escherichia coli* (EPEC) refers to a group of *E. coli* serotypes that was once an important cause of outbreaks of diarrhea among infants in hospitals and nurseries in Europe and North America (10, 15, 19). While outbreaks due to EPEC appear to have declined in these countries, EPEC persists as a major bacterial cause of diarrhea in the developing countries. EPEC has been recognized as the most frequent bacterial cause of diarrhea in children less than 1 year of age in the urban centers of Brazil, Nigeria, and Mexico (1, 8, 31). However, despite its recognized clinical and epidemiologic importance, the mechanisms of virulence of EPEC, when compared with those of enterotoxigenic *E. coli* or enteroinvasive *E. coli*, are still poorly understood.

Ultrastructural studies of intestinal biopsy specimens from patients with EPEC-induced diarrhea show that EPEC attach to the intestinal epithelium in a characteristic fashion and cause effacement of the microvilli, features that may be critical in the pathogenesis of diarrhea (27, 33). The bacterial products that mediate these events, however, are unknown. Recently, several investigators have demonstrated that some *E. coli* belonging to the classical EPEC group, but not those belonging to the enterotoxigenic, enteroinvasive, or enterohemorrhagic groups, attach to human laryngeal (HEp-2) or cervical (HeLa) tissue culture cells in clusters; this pattern of attachment has been termed localized adherence (LA) (6, 7, 29). Baldini et al. have shown that LA is plasmid mediated. EPEC adherence factor has been proposed by these investigators to refer to the putative adhesin responsible for LA (2). More recently, Baldini et al. (2) have localized the genetic determinant of EPEC adherence factor to a 60-megadalton (MDa) plasmid in an O127:H6 EPEC strain (3). However, so far no gene product of the plasmid has been identified. In this investigation, we found that a 54-MDa plasmid not only encodes LA in an O111:NM EPEC strain, but also is required for the expression of the O111-specific polysaccharide side chain of the lipopolysaccharide (LPS). Recent reports have documented the plasmid-mediated LPS O antigen expression in some *Shigella* species (11, 12, 17, 28, 34, 35) and some *Salmonella* serotypes (26). These observations suggest that the O-specific polysaccharides of some enteric pathogens may play a role in the characteristic virulence activity of the organism and that plasmids may be involved in LPS modification in more enteric bacterial pathogenic species than previously recognized.

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MATERIALS AND METHODS

Bacterial strains. An O111:NM *E. coli* strain (B171) isolated from the stool of a child in a recent outbreak of diarrhea in a day care center (25) was kindly provided by I. K. Wachsmuth, Enteric Diseases Laboratory, Centers for Disease Control, Atlanta, Ga. Strain B171 was resistant to chloramphenicol, tetracycline, streptomycin, and sulfathiazole. It hosted three plasmids; a 54-MDa plasmid was designated pYR111. A derivative strain, designated B171-4, was constructed from B171 by elimination of pYR111. A nalidixic acid-resistant derivative of *E. coli* HB101 (F^- *hsdS20* [r_k m_k] *recA13 ara-14 proA2 lacxY1 galK2 rpsL20* [Sm^r] *xyl-5 mtl-1 supE44*) (kindly provided by Stanley Falkow, Stanford University) was used as a recipient strain in conjugation experiments.

Plasmid DNA isolation and curing procedures. The bacterial strains were grown overnight in 2 ml of Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) containing chloramphenicol (0.02 mg/ml), and the plasmid DNA was extracted and precipitated from lysed cells by a modified version of a method described by Birnboim and Doly (4). The DNA was electrophoresed in 0.8% agarose gels and stained with ethidium bromide, and the gel was photographed under UV illumination.

We cured plasmid pYR111 in strain B171 by growing the bacteria in a subinhibitory concentration of novobiocin (Sigma Chemical Co., St. Louis, Mo.) by the method of McHugh and Swartz (22). Plasmid DNA, extracted from novobiocin-treated bacteria plated onto Trypticase soy agar for single-colony isolation, was analyzed by agarose gel electrophoresis.

Conjugation procedures. The bacterial strains were grown

at 37°C in TSB overnight. Transfer of pYR111 was promoted by mixing 0.3 ml each of the overnight growth of donor (B171) and recipient (HB101) strains with 4 ml of TSB over Trypicase soy agar in a plate and incubating the mixture for 3 h at 37°C. The mating mixture was then plated onto MacConkey agar (Difco Laboratories, Detroit, Mich.) containing chloramphenicol (0.02 mg/ml) and nalidixic acid (0.05 mg/ml). The donor and recipient strains were also plated separately on selective media as controls. Lactose-negative, chloramphenicol-resistant, nalidixic acid-resistant colonies growing on the MacConkey agar were picked and examined for their plasmid content.

LPS isolation and immunoautoradiography. LPS from selected bacterial strains was extracted and purified by a procedure described by Westphal and Jann (36). LPS from the transconjugants was identified by the proteinase K digestion procedure of Hitchcock and Brown (13). The LPS was analyzed by electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel (15% running gel containing 4 M urea and 5% stacking gel with no urea) and visualized by silver staining as described by Tsai and Frasch (32).

To test the antigenic reactivity of the O side chains with O-specific antisera, the LPS resolved by polyacrylamide gel electrophoresis was electroeluted onto nitrocellulose by a modification of a procedure described by Bradbury et al. (5). The nitrocellulose paper with the transblotted LPS was incubated overnight at 37°C in 200 ml of 10 mM Tris hydrochloride buffer (pH 7.4) containing 0.15 M NaCl and 3.5% (wt/vol) gelatin. The paper was then incubated at 37°C for 1.5 h in O111-specific rabbit antiserum (kindly provided by I. K. Wachsmuth) diluted 1:400 or 1:800 in the same Tris-saline buffer containing 1% (wt/vol) bovine serum albumin instead of gelatin. The paper was then washed in Tris-saline buffer containing 0.5% Brij 35 (Sigma), incubated at 37°C for 1 h in 25,000 cpm of ^{125}I-protein A (Amersham Corp., Arlington Heights, Ill.) per ml, washed again with the Tris-saline-Brij buffer, and exposed to AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) at −70°C.

Biochemical analysis of the LPS carbohydrate. The O-antigenic unit of the O111 *E. coli* LPS is composed of five monosaccharides, with a unique hexose residue, 3,6-dideoxyhexose (colitose), linked 1→3 and 1→6 to α-D-glucose (9). Therefore quantitative determination of the O111 polysaccharide in the extracted LPS was made by detecting 3,6-dideoxyhexose in the purified samples of LPS heated in periodic acid as described previously (16). The 3,6-dideoxyhexose content was quantitatively expressed as the molar ratio of this monosaccharide to the total heptose content detected in the LPS samples.

HeLa cell binding assay. The HeLa tissue culture cell adherence assay was performed by a procedure modified from the method of Scaletsky et al. (29). The HeLa cell monolayer in a 24-well polystyrene tissue culture flask containing 1.5 ml of Eagle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) per well, 10% fetal calf serum, and 0.5% D-mannose was incubated for 1.5 h at 37°C with 0.1 ml of overnight bacterial growth in TSB. Mannose was included to inhibit the binding to HeLa cells of bacteria expressing type 1 fimbriae. Each well was then washed six times with phosphate-buffered saline (pH 7.3) and refilled with fresh Eagle minimal essential medium, and the plate was incubated for an additional 1.5 h at 37°C. The monolayer in the wells was then washed three times with phosphate-buffered saline, fixed with methanol, stained with 10% Giemsa stain, and examined under an inverted-light microscope for the characteristic pattern of attachment.

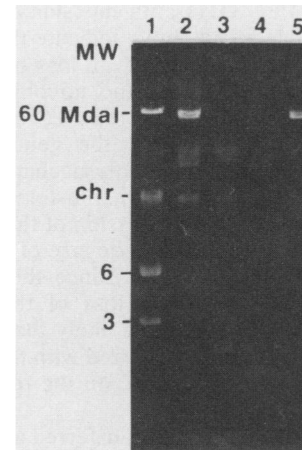

FIG. 1. Agarose gel electrophoresis of plasmid DNA showing the results of plasmid-curing and conjugation experiments. Lanes: 1, molecular weight (MW) markers in megadaltons; 2, *E. coli* B171 harboring a 54-MDa plasmid designated pYR111 and two smaller plasmids of less than 3 MDa; 3, derivative strain B171-4, showing the loss of pYR111 but the retention of the two smaller plasmids; 4, plasmid-free, nalidixic acid-resistant derivative of *E. coli* HB101; 5, *E. coli* HB101(pYR111) transconjugant, indicating the acquisition of pYR111 but not of the other plasmids. Abbreviation: chr, chromosomal DNA.

RESULTS

Effect of eliminating pYR111 on the phenotypic characteristics of strain B171. Baldini et al. have recently shown that most EPEC strains that adhere to HeLa or HEp-2 cells in clusters possess 50- to 70-MDa plasmids (2). The O111:NM EPEC strain (B171) used in this study harbored a 54-MDa plasmid (pYR111) in addition to two smaller plasmids of less than 3 MDa (Fig. 1). At a subinhibitory concentration of novobiocin (0.3 mg/ml), a derivative strain cured of pYR111 (B171-4) was identified among the first 50 novobiocin-treated strains examined. Upon elimination of pYR111, the EPEC derivative strain failed to exhibit localized adherence to HeLa cells, lost resistance to chloramphenicol, tetracycline, streptomycin, and sulfathiazole, and, when propagated in TSB, clumped to the bottom of the tube, indicative of rough bacterial strains that lack the hydrophilic LPS O-polysaccharide side chains. Strain B171 was reconfirmed at the Centers for Disease Control as an O111 serogroup *E. coli*, and strain B171-4 was reported untypable.

To demonstrate that strain B171-4 lacked the O-side chain repeat units, we analyzed the extracted LPS from strains B171 and B171-4 by SDS-polyacrylamide gel electrophoresis (Fig. 2). The multimeric repeating units of LPS O polysaccharide resolved as ladderlike bands in an SDS-polyacrylamide gel. The extracted LPS from strain B171-4, in contrast to that from strain B171, lacked such regularly repeating O side chains (Fig. 2, lane 3). Instead, low-molecular-weight bands indicative of the lipid A core polysaccharide were observed.

The above finding was confirmed by the quantitative determination of the O111-specific monosaccharide, 3,6-dideoxyhexose, in LPS extracted from strains B171 and B171-4. The LPS from strain B171 contained 1.28 μmol of 3,6-dideoxyhexose per μmol of heptose, whereas this monosaccharide was not detected in LPS from strain B171-4.

Expression of EPEC O111-antigenic side chains in *E. coli* HB101. The data described above indicate that the plasmid-curing procedure was associated with loss of O side chains. However, it was possible that the novobiocin induced a chromosomal mutation that affected LPS biosynthesis. To determine that pYR111 carried the genes required for expression of the O111-specific polysaccharide, it was necessary to show that the plasmid, transferred into another bacterial host, could confer expression of the O111 polysaccharide by that host. Because of the size of pYR111, it was assumed to be self-transferrable. Since the elimination of pYR111 from B171 resulted in loss of the antimicrobial resistance of the strain, it seemed likely that the antimicrobial resistance would be cotransferred with the plasmid, thus conferring a selectable phenotype on the plasmid recipient strains.

Antimicrobial resistance was transferred at a frequency of about 10^{-9} transconjugants per donor cell. All but 2 of 100 tested nalidixic acid- and chloramphenicol-resistant transconjugants were also resistant to streptomycin, sulfathiazole, and tetracycline. The other two transconjugants lacked resistance to tetracycline. The transfer of pYR111 to these transconjugants was confirmed by direct visualization of the plasmid by agarose gel electrophoresis as described previously (Fig. 1). Eighteen of these transconjugants contained, in addition to the 54-MDa plasmid, the lower-molecular-weight plasmids of strain B171. These transconjugants were excluded from further experiments.

Of 32 tested transconjugants, 28 showed LA with HeLa cells. However, the clusters of bacteria on the HeLa cells were smaller than the clusters of strain B171.

The method of LPS extraction by proteinase K digestion of whole bacterial cells facilitated the screening of a large number of transconjugants for strains that expressed the O side chains. The deproteinized lysates of 15 of 43 tested transconjugants were shown by SDS-polyacrylamide gel electrophoresis and silver staining to express the O-polysaccharide side chains. O-polysaccharide expression by the transconjugants appeared to depend on growth conditions: expression was considerably reduced when the bacteria were grown in liquid instead of on solid medium.

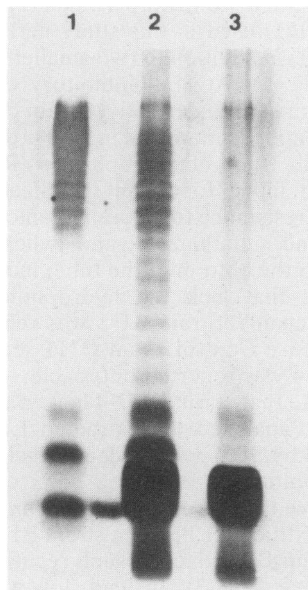

FIG. 2. Electrophoretic separation in a polyacrylamide gel of LPS extracted from *E. coli* serotype O111:B4 obtained commercially (Sigma) (lane 1), *E. coli* O111:NM (B171) (lane 2), and derivative strain B171-4 (lane 3). The LPS from the last two strains was extracted by the method of Westphal and Jann (36). Lanes 1 and 2 show regularly spaced bands representing LPS molecules with increasing numbers of O side chain repeat units. The LPS extract from the plasmid-cured derivative of B171 (lane 3) yielded only low-molecular-weight bands indicative of the lipid A core polysaccharide, e.g., the rough LPS phenotype.

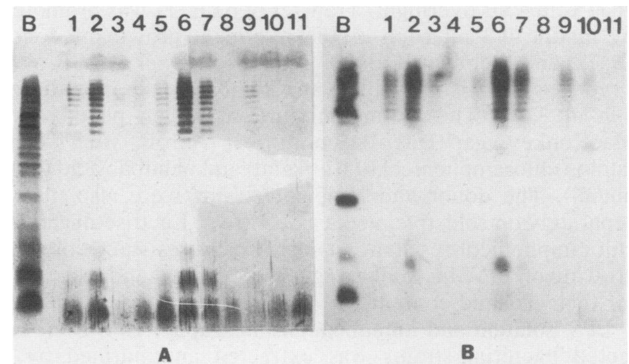

FIG. 3. Electrophoretic and antigenic analysis of LPS extracted by proteinase K digestion of *E. coli* B171 (lane B) and HB101 (lane 11), and of HB101(pYR111) transconjugants (lanes 1 to 10). The same SDS-polyacrylamide gel was used for siver staining (panel A) and for immunoblotting (panel B) with O111-specific polyclonal antiserum. B171 and 9 of 10 transconjugants exhibit regularly spaced bands of increasing molecular weight that are recognized by O111 antiserum, indicating the expression of the O111 antigen by these strains.

To show that the repeating side chains expressed by HB101(pYR111) transconjugants exhibited the O111-specific antigen of the EPEC strain, it was necessary to show that the side chain units were bound by the O111-specific antiserum. This was accomplished by using the immunoblotting method described previously. The O-polysaccharide side chains expressed by HB101(pYR111) strains cross-react with the O111-specific antiserum (Fig. 3).

DISCUSSION

That LA represents a unique plasmid-mediated virulence property of EPEC is suggested by both volunteer and field studies (21; J. J. Mathewson, R. A. Oberhelman, H. L, Dupont, B. E. Murray, F. J. Dela Cabada, and E. V. Garibay, Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 48, 1986). So far, however, the specific determinants of LA have not been identified. In the present investigation, we found that the plasmid that encodes LA by an O111:NM EPEC strain also specifies drug resistance to four antibiotics and the expression of the LPS O antigen. The occurrence of antimicrobial resistance genes on the LA plasmid has been reported recently from Brazil (18), but, as far as is known, plasmid-encoded LPS O-polysaccharide expression by EPEC or any *E. coli* strain has not been reported.

The evidence that the O-polysaccharide side chain of strain B171 is encoded by pYR111 is supported by the findings that (i) the elimination of pYR111 resulted in the irreversible loss of O-polysaccharide expression by strain B171 and (ii) the transfer of pYR111 to *E. coli* HB101 conferred to the new host the ability to express the O111-

specific antigen. This study, however, did not determine whether pYR111 encodes factors that regulate chromosomal determinants of the O111 side chain expression or enzymes that directly mediate O111 side chain biosynthesis.

The basic LPS structure of a gram-negative bacterium is usually specified by clusters of genes located in the chromosome. However, it has been recognized for many years that with some bacteria these basic structures can be modified by extrachromosomal elements such as bacteriophage genomes (20). More recently, Kopecko et al. noted that a 120-MDa plasmid, a virulence plasmid necessary for HeLa cell invasion, encodes the expression of the form I antigen that makes up the O-polysaccharide side chain of *Shigella sonnei* (17). Watanabe and Timmis reported that in *Shigella dysenteriae* a 6-MDa plasmid is necessary for virulence as well as somatic antigen expression (35). Hale et al. observed that the expression of the *S. dysenteriae* 1 somatic antigen in *E. coli* K-12 depends both on the 6-MDa plasmid and on a chromosomal region cotransducible with the his^+ marker from *S. dysenteriae* 1 (11). Popoff and LeMinor have reported that the O-polysaccharide antigen of a *Salmonella* strain in group O54 was determined by a plasmid of 5 MDa (26). These observations, together with the findings from this present investigation with *E. coli*, suggest that plasmid-determined LPS O-antigen expression in enteric bacterial pathogens may be more common than was previously recognized.

The loss of the capacity by strain B171 to exhibit LA concurrently with the loss of O side chain expression raises a question concerning the role of the LPS O antigen in adherence. Izhar et al. have shown that LPS O polysaccharides of *Shigella flexneri* can competitively inhibit the attachment of this bacteria to guinea pig colonic mucosa (14), suggesting that the O antigen mediates mucosal adhesion. At present we do not know what role the O111 antigen plays in the interaction of the EPEC strain B171 with HeLa cells, and we do not know whether such plasmid-specified O-antigen expression occurs in other serogroups of EPEC that exhibit LA. Orskov et al. (24) examined O antigens extracted from various *E. coli* serotypes and found that strains representing the classical EPEC serotypes, including O111, could be classified according to a characteristic immunoelectrophoretic migration pattern of their O antigens that is distinct from the pattern observed for the O antigens from *E. coli* serotypes associated with dysenterylike illness. We do not know whether this feature of O antigens of EPEC contributes to the initial interaction of the bacteria with eucaryotic cells that may lead to the intimate attachment of the bacteria involving characteristic pedestallike projections of the enterocyte and effacement of the microvilli. The pathogenic role of the O-polysaccharide antigen of strain B171 is currently under investigation.

The occurrence of genetic determinants of a characteristic virulence mechanism such as enterocyte invasion or epithelial cell adherence on the same plasmid that encodes or regulates a specific O-antigen expression raises an intriguing hypothesis that may explain the long-recognized classification of *E. coli* that cause diarrhea according to a pathogenic mechanism (i.e., enterotoxigenicity, enteroinvasion, and enteroadherance) and corresponding distinct serogroups (23, 30). If the genes encoding a group of LPS O antigens are always cotransferred with the unique virulence genes of the *E. coli*, an *E. coli* strain that exhibits a characteristic pathogenic activity would belong to a specific serogroup. Detailed studies of this class of plasmids encoding LPS modification may provide new insights into the bacterial pathogenesis of diarrhea.

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LITERATURE CITED

1. **Agbonlahor, D. E., and T. O. Odugbemi.** 1982. Enteropathogenic, enterotoxigenic and enteroinvasive *E. coli* isolated from acute gastroenteritis patients in Lagos, Nigeria. Trans. R. Soc. Trop. Med. Hyg. **76**:265–267.
2. **Baldini, M. M., J. B. Kaper, and M. M. Levine.** 1983. Plasmid-mediated adhesion in enteropathogenic *Escherichia coli*. J. Pediatr. Gastroenterol. Nutr. **2**:534–536.
3. **Baldini, M. M., J. P. Nataro, and J. B. Kaper.** 1986. Localization of a determinant for HEp-2 adherence by enteropathogenic *Escherichia coli*. Infect. Immun. **52**:334–336.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
5. **Bradbury, W. C., S. D. Mills, M. A. Preston, L. J. Barton, and J. L. Penner.** 1984. Detection of lipopolysaccharides in polyacrylamide gels by transfer to nitrocellulose followed by immunoautoradiography with antibody and 125-I-protein A: "LPS blotting". Anal. Biochem. **137**:129–133.
6. **Clausen, C. R., and D. C. Christie.** 1983. Chronic diarrhea in infants caused by adherent enteropathogenic *Escherichia coli*. J. Pediatr. **100**:358–361.
7. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3**:95–99.
8. **Donta, S. T., R. B. Wallace, S. C. Whipp, and J. Olarte.** 1977. Enterotoxigenic *Escherichia coli* and diarrheal disease in Mexican children. J. Infect. Dis. **135**:482–485.
9. **Edstrom, R. D., and E. C. Heath.** 1967. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. VII. Studies on the structure of the O-antigenic polysaccharide. J. Biol. Chem. **242**:4125–4133.
10. **Giles, C., and M. B. Sangster.** 1948. An outbreak of infantile gastroenteritis in Aberdeen. J. Hyg. **46**:1–9.
11. **Hale, T. L., P. Guerry, R. C. Seid, C. Kapfer, M. E. Wingfield, C. B. Reaves, L. S. Baron, and S. B. Formal.** 1984. Expression of lipopolysaccharide O-antigen in *Escherichia coli* K-12 hybrids containing plasmid and choromosomal genes from *Shigella dysenteriae* 1. Infect. Immun. **46**:470–475.
12. **Hale, T. L., P. J. Sansonetti, P. A. Schad, S. Austin, and S. B. Formal.** 1983. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. Infect. Immun. **40**:340–350.
13. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
14. **Izhar, M., Y. Nuchamoiwitz, and D. Mirelman.** 1982. Adherence of *Shigella flexneri* to guinea-pig intestinal cells is mediated by a mucosal adhesin. Infect. Immun. **35**:1110–1118.
15. **Jacobs, S. I., A. Holzel, B. Wolman, J. H. Keen, V. Miller, J. Taylor, and R. J. Gross.** 1970. Outbreak of infantile gastroenteritis caused by *Escherichia coli* O114. Arch. Dis. Child. **45**:656–663.
16. **Keleti, G., and W. H. Lederer.** 1974. Handbook of micromethods for the biological sciences, p. 57–58. Van Nostrand Reinhold Co., New York.
17. **Kopecko, K. J., O. Washington, and S. B. Formal.** 1980. Genetic and physical evidence for plasmid control of *Shigella sonnei*

form I cell surface antigen. Infect. Immun. **29**:207–214.
18. **Laposta, M. Z., M. L. M. Silva, I. C. A. Scaletsky, and L. R. Trabulsi.** 1986. Plasmids coding for drug resistance and localized adherence to HeLa cells in enteropathogenic *Escherichia coli* O55:H$^-$ and O55:H6. Infect. Immun. **51**:715–717.
19. **Laurell, G., J. H. Magnusson, E. Frisell, and B. Werner.** 1951. Epidemic infantile diarrhea and vomiting. Acta Paediatr. Scand. **40**:302–337.
20. **Lederberg, J.** 1955. Recombination mechanisms in bacteria. J. Cell. Comp. Physiol. **45**:75–107.
21. **Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, J. B. Kaper, R. E. Clements, and A. D. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on plasmid encoding enteroadhesiveness factor. J. Infect. Dis. **152**:550–558.
22. **McHugh, G. L., and M. N. Swartz.** 1977. Elimination of plasmids from several bacterial species by novobiocin. Antimicrob. Agents Chemother. **12**:423–426.
23. **Orskov, F., I. Orskov, D. J. Evans, R. B. Sack, D. A. Sack, and T. Wadstrom.** 1976. Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. Med. Microbiol. Immunol. **162**:73–80.
24. **Orskov, F., I. Orskov, B. Jann, and K. Jann.** 1971. Immunoelectrophoretic patterns of extracts from all *Escherichia coli* O and K antigen test strains, correlation with pathogenicity. Acta Pathol. Microbiol. Scand. **79**:142–152.
25. **Paulozzi, L. J., K. E. Johnson, L. M. Kamahele, C. R. Clausen, L. W. Riley, and S. D. Helgerson.** 1986. Diarrhea associated with adherent enteropathogenic *Escherichia coli* in an infant and toddler center, Seattle, Washington. Pediatrics **77**:296–300.
26. **Popoff, M. Y., and L. Le Minor.** 1985. Expression of antigenic factor O54 is associated with the presence of a plasmid in Salmonella. Ann. Inst. Pasteur Microbiol. **136b**:169–179.
27. **Rothbaum, R., A. J. McAdams, R. Gianella, and J. C. Partin.** 1982. A clinicopathologic study of enterocyte-adherent *Escherichia coli*: a cause of protracted diarrhea in infants. Gastroenterology **83**:441–454.
28. **Sansonetti, P. J., T. L. Hale, G. J. Dammin, C. Kapfer, H. H. Collins, and S. B. Formal.** 1983. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. Infect. Immun. **39**:1392–1402.
29. **Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi.** 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. **45**:534–536.
30. **Stenderup, J., and F. Orskov.** 1983. The clonal nature of enteropathogenic *Escherichia coli* strains. J. Infect. Dis. **148**:1019–1024.
31. **Toledo, M. R. F., C. C. B. Alvariza, J. Murahovschi, R. T. S. Ramos, and L. R. Trabulsi.** 1983. Enteropathogenic *Escherichia coli* serotypes and endemic diarrhea in infants. Infect. Immun. **39**:586–589.
32. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
33. **Ulshen, M. H., and J. L. Rollo.** 1980. Pathogenesis of *Escherichia coli* gastroenteritis in man—another mechanism. N. Engl. J. Med. **302**:99–101.
34. **Watanabe, H., A. Nakamura, and K. N. Timmis.** 1984. Small virulence plasmid of *Shigella dysenteriae* 1 strain W30864 encodes a 41,000-dalton protein involved in formation of specific lipopolysaccharide side chains of serotype 1 isolates. Infect. Immun. **46**:55–63.
35. **Watanabe, H., and K. N. Timmis.** 1984. A small plasmid in *Shigella dysenteriae* 1 specifies one or more functions essential for O-antigen production and bacterial virulence. Infect. Immun. **43**:391–396.
36. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–91.