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Bacterial Clump Formation at the Surface of Liquid Culture as a Rapid Test for Identification of Enteroaggregative *Escherichia coli*

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Forty-one strains of enteroaggregative *Escherichia coli* formed clumps visible as a scum at the surface of a Mueller-Hinton broth shaker culture. Sixty-one control strains of *E. coli* did not. Scum formation is a simple, rapid, and inexpensive test for the identification of enteroaggregative *E. coli*.

The four well-recognized classes of *Escherichia coli* that cause diarrhea are enteropathogenic, enterotoxigenic, enteroinvasive, and enterohemorrhagic *E. coli* (6). Recently, a fifth category of *E. coli*, recognizable by its aggregative or "stacked-brick" type of adherence to cultured mammalian cells, has been recognized as yet another category of diarrheagenic *E. coli* in children in different parts of the world (2, 3, 5, 8). Because of its characteristic aggregative type of adherence, this *E. coli* has been referred to as enteroaggregative *E. coli* (EAggEC) (2, 7).

The diagnosis of EAggEC is dependent upon a cell culture adherence assay (7) or a DNA probe assay (1), both of which are expensive and cumbersome and are beyond the reach of the majority of clinical laboratories. Recently, Yamamoto et al. (9) observed that a strain of EAggEC, TL100 (serotype O127a:H2), isolated from a child with diarrhea in Thailand formed bacterial clumps visible as a thick scum in a liquid culture. We screened EAggEC obtained from various geographical locations in an attempt to find out whether bacte-

TABLE 1. Bacterial clumping or scum formation at surface of MHB shaker culture after 20 h of incubation at 37°C

Bacteria	No. of strains tested	Source[a]	Bacterial clump formation[b]
EAggEC strains (serotypes) 950C2, 66C1, 518C3, 66C3 (O113:H−), 180C3, 950C3 (O113:H27), 130C2, 1429C2, 300C3, 1210C2, 186C1, 779C2, 779C1 (O141:H49), 396C1, 1298C3, 522C1, 634C1	17	K. Haider, Bangladesh	+
75525 (O73:H18), 75527 (O?:H13), 75530 (O134:H27), 75532 (O86:H18), 75535 (O?:H27)	5	M. Penny, Peru	+
221 (O92:H33)	1	M. Levine, United States	+
F01, F02, F03, F04, F05, F06, H07, H08	8	M. K. Bhan, India	+
W6-1-5 (O?:H2), W44-1-3 (O4,36:H18), W253-1-1 (O3:H2), W309-1-1 (O130:H27), W365-1-5 (O17:H−), WC83-1-2 (O15:H18), WC99-1-5 (O38:H9), WC232-1-3 (O127:H21), WC310-1-1 (O?:H21), WC354-1-3 (O86:H19)	10	P. Echeverria, Thailand	+
Enterotoxigenic *E. coli*	10	Stock culture	−
Enteropathogenic *E. coli*	10	Stock culture	−
Enteroinvasive *E. coli*	10	Stock culture	−
Enterohemorrhagic *E. coli*	10	Stock culture	−
Diffuse adherent *E. coli*	10	Stock culture	−
Nonpathogenic *E. coli*	10	Stock culture	−
E. coli K-12	1	Nonpathogenic laboratory strain	−

[a] Bangladeshi strains were isolated at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Peruvian strains were isolated at the Institute of Investigacion Nutricional, Lima, Peru. Indian strains were isolated at the All India Institute of Medical Sciences, New Delhi, India. Thai strains were isolated at the Armed Forces Medical Research Institute, Bangkok, Thailand. The U.S. strain was isolated from an American tourist in Mexico. The remaining strains were from the ICDDR, B culture collection.

[b] +, positivity for all strains; −, negativity for all strains.

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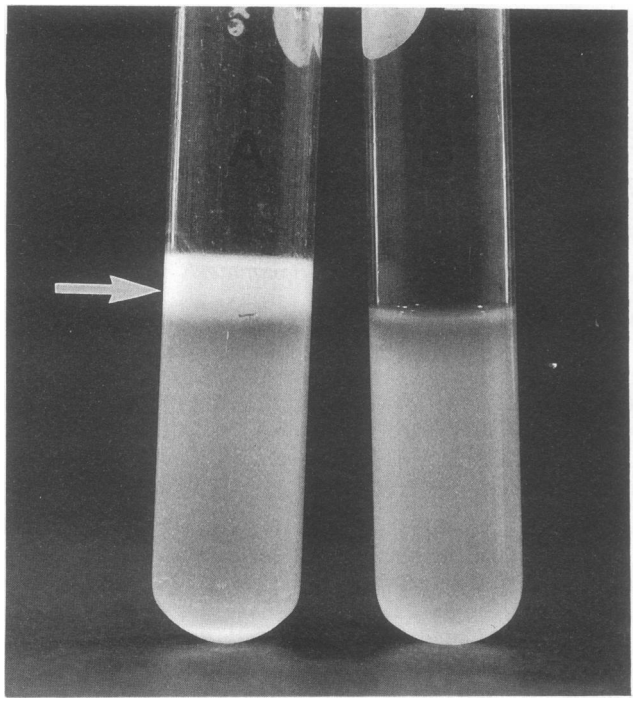

FIG. 1. Clump formation visible as a scum (indicated by arrow) by an EAggEC strain in tube A and no clump formation by a negative control *E. coli* strain in tube B (what appears as a thin ring at the top of the liquid culture in tube B is due to a reflection of light). The cultures were 20-h-old MBH shaker cultures.

rial clump formation in a liquid culture could be used as a simple and rapid test for the identification of these bacteria.

The bacterial strains tested blindly for scum formation and their sources are indicated in Table 1. All of the EAggEC strains, except for five strains from Thailand (indicated by the prefix WC), originated from children with diarrhea. The five Thai strains with the prefix WC were isolated from healthy children. All of the strains were confirmed as EAggEC by a HEp-2 cell adherence assay (5) and a DNA probe assay (1). The control cultures were from the culture collection of the International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh. Enterotoxigenic, enteropathogenic, enteroinvasive, and enterohemorrhagic *E. coli* were identified as described previously (4). Nonpathogenic *E. coli* isolated from healthy individuals did not possess any of the virulence properties of diarrheagenic *E. coli*.

Initial studies were done with six strains of EAggEC, namely, strains 221, 66C1, F01, 180C3, 75527, and WC309-1-1, to find out the optimum conditions for scum formation. Optimal conditions were investigated with Mueller-Hinton broth (MHB) (Difco Laboratories, Detroit, Mich.), nutrient broth (3 g of Difco beef extract, 5 g of Difco peptone, and 8 g of NaCl, all in 1 liter of distilled H_2O) (pH 7.4), and Luria broth (10 g of Difco tryptone, 5 g of Difco yeast extract, and 5 g of NaCl, all in 1 liter of distilled H_2O) (pH 7.5). Organisms were subcultured on MacConkey agar, and then the growth was inoculated into 5 ml of each of three media in duplicate tubes. One set of tubes was incubated in the stationary position at 37°C, and the other set was incubated in an orbital shaker incubator (Gallenkamp, Leicestershire, United Kingdom) adjusted to 100 rpm for 20 h. Bacterial clumps visible as a scum at the surface of the liquid culture were noted at the end of the incubation (Fig. 1). All of the organisms produced scum varying in breadth from 0.5 to 1.0 cm in MHB and Luria broth in both stationary and shaker cultures. However, they did not produce scum in nutrient broth, in either a stationary or a shaker culture. This is contrary to the observations of Yamamoto et al. (9), who found that their strain, TL100, also formed scum in nutrient broth. It is likely that differences in the quality of media would have contributed to the ability to support scum formation. Light microscopy of an unstained preparation of scum revealed large bacterial clumps. Even after vigorous shaking, bacterial clumps remained as a ring on the side of the test tube. Scum formation was best in the MHB shaker culture, as judged by the scum's thickness and ability to stick to the test tube as a ring. On the basis of these preliminary studies, a 20-h MHB shaker culture at 37°C was used for screening all isolates.

All 41 EAggEC isolates produced scum, and none of the other 61 control *E. coli* isolates did so (Table 1). Thus, there was a 100% correlation between scum formation and the aggregative pattern of adherence of *E. coli*. The serotypes of 19 EAggEC isolates were known, and these serotypes were different. This suggested that scum formation is not dependent upon serotype. Therefore, scum formation promises to be a simple, rapid, accurate, and inexpensive test for the identification of EAggEC. However, the ability of the test should be evaluated further with fresh clinical isolates.

Preliminary evidence suggests that the aggregative adherence property is mediated by an ~60-MDa plasmid, and the DNA probe used for the identification of EAggEC is also derived from one such plasmid (1). Moreover, Yamamoto et al. (9) found that insertional inactivation of the plasmid conferring aggregative adherence with transposon Tn*1* in the strain TL100 resulted in the loss of bacterial clump formation. This suggests that clump formation is also mediated by this plasmid.

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