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LETTER TO THE EDITOR

RELIABILITY OF COLONY CHARACTERISTICS ON MacCONKEY AGAR TO IDENTIFY *ESCHERICHIA COLI* FOR EPIDEMIOLOGICAL INVESTIGATIONS

Sir:

Escherichia coli form the predominant facultative bacterial flora of the faeces in most individuals (1). Outside their normal habitat of mainly the large intestine, some strains are pathogenic, for example, those which produce septicaemia, wound infections, urinary tract infections, and meningitis (2). Moreover, even within the intestinal tract, certain categories of *E. coli* are pathogenic, mainly producing diarrhoea. Recent studies have shown that there are at least 5 categories of diarrhoeagenic *E. coli*: enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli* (3) and enteroaggregative *E. coli* (4) and they produce disease by different mechanisms. In epidemiological studies on the role of diarrhoeagenic *E. coli*, it is a common practice to pick up three or more lactose-fermenting colonies resembling those of *E. coli* from MacConkey agar in the assumption that they are *E. coli*, and to test them for various diarrhoeagenic properties (5,6,7,8). However, the reliability of this practice has never been documented. There are several other members within the family *Enterobacteriaceae* present in the faeces that can easily resemble *E. coli* (1). Moreover, there are at least 4 different species of organisms within the genus *Escherichia* that have been recently described, and at least one other lactose-fermenting species, viz. *E. hermanii*, can also be present in human faeces (9). Therefore, the possibility exists that a lactose-fermenting colony resembling *E. coli* could also be at least *E. hermanii* or any lactose-fermenting organism from other genera of *Enterobacteriaceae*. This study was undertaken to find out how far the practice of identifying *E. coli* on the basis of colony characteristics on a primary faecal culture plate of MacConkey agar is reliable for the correct identification of the organism. This is important for the accurate diagnosis of *E. coli* diarrhoea specially for epidemiological investigations.

Single diarrhoeal stool specimens from 340 patients of all age groups seeking treatment at the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, between December 1989 and February 1990 were cultured for enteric pathogens by standard methods (1,2). Stools were inoculated onto a battery of media including MacConkey agar (Oxoid Laboratories, England). After overnight incubation at 37°C, three individual pink colonies typical of *E. coli* (non-mucoid colonies either smooth and convex or corrugated with spreading edge, measuring in diameter from approximately 2.5 to 5 mm) were picked up from MacConkey agar and screened biochemically for their correct identities. Preliminary screening was done by inoculating Kligler's iron agar and motility-indole-urea medium (10) and were further screened by inoculating KCN broth and sorbitol and glycerol sugars, for differentiation of the organisms within the genus *Escherichia* (9). Further screening with biochemical strip API 20E (API system, Montalieu, Vercieu, France) was carried out, when required, for accurate identification of colonies.

The identification of colonies based on their biochemical reactions is shown in the Table. Approximately 96% of the colonies picked up as *E. coli* on the basis of colony characteristics were confirmed as *E. coli* by biochemical reactions. However, no species other than *E. coli* was encountered within the

BIOCHEMICAL IDENTITIES OF 1020 COLONIES PICKED UP AS *E. COLI* FROM MacCONKEY AGAR CULTURED FROM STOOLS OF 340 PATIENTS WITH DIARRHOEA

Organisms	n = 1020 (%) of colonies
Escherichia coli	976 (95.7)
Citrobacter freundii	16 (1.6)
Klebsiella pneumoniae	16 (1.6)
Enterobacter cloacae	6 (0.6)
Enterobacter agglomerans	4 (0.4)
Citrobacter amalonaticus	1 (0.1)
Serratia rifera	1 (0.1)

genus *Escherichia*. Only a minor proportion of colonies were identified as members of other genera within the family *Enterobacteriaceae*. Our results, thus, suggest that the practice of identifying *E. coli* on the basis of colony characteristics on MacConkey agar is satisfactory for epidemiological investigations. However, whenever absolute accuracy is required, all colonies should be biochemically screened. Moreover, our study was confined to 3 winter months; perhaps, extension of the study to other seasons and countries might reveal some variation in the result.

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