

ISOLATION OF HEAT-STABLE ENTEROTOXIN-PRODUCING STRAINS OF ESCHERICHIA COLI ISOLATED FROM CASES OF DIARRHEA IN CAMPINAS, SP, BRAZIL

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S U M M A R Y

The Authors report the occurrence of three strains of *Escherichia coli* producing only toxin of the ST type, revealed by DEAN'S test. When tested in Y1 cells and in rabbit skin, these strains showed no production of LT toxin. In all cases the ST-producing strains provided an intestine to body weight ratios equal or greater than 0.122. On the other hand, 21 ST-negative strains produced ratios between 0.050 and 0.059 followed by 15 strains which gave values between 0.060 and 0.069. Only two negative strains evoked reactions between 0.070 and 0.075.

I N T R O D U C T I O N

Diarrheal disease is one of the leading causes of death among children under three years of age, who live in developing countries⁷.

Bacteriological examinations of diarrheal stools in different countries have often given similar results, characterized by the absence, in many cases, of the known recognized enteropathogens.

Since 1956, after DE et al.¹ it has become clear that some colibacilli, not necessarily belonging to the classic enteropathogenic serotypes, were able to cause dilatation and increased volumes of fluid in the ligated intestinal ileal loop test performed in adult rabbits.

Later, other Authors^{5,8,9}, besides confirming these findings, found that this picture could be reproduced with bacteria-free filtrates of known *E. coli* strains which had been isolated from pigs and calves with diarrhea and had been found to produce dilatation

when injected into the ligated intestine as live bacterial cultures. Based upon these experiments it was concluded that at least in those cases enteropathogenicity was due to the production of an enterotoxin.

Human and swine enteropathogenic *Escherichia coli* are now known to produce at least two types of enterotoxin. One, heat labile (LT), similar, if not identical, to cholera toxin, with high molecular weight, is able to give positive results in the adult rabbit ileal loop⁷. As it occurs with cholera toxin, supernatants either concentrated or not of these *E. coli* strains when inoculated intradermally in the rabbit depilated skin caused localized induration and increased permeability of small blood vessels. This property of LT toxin of *E. coli*, named permeability factor (PF) and first observed with cholera toxin, may be used for its characterization and measurement⁶.

Recently it has been demonstrated that bacteria-free filtrates of these microorga-

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nisms were able to cause rounding of the elongated Y1 adrenal cells³. This effect was not anymore observed when these preparations were previously heated at 100°C for 15 minutes.

On account of several experiments carried out first with *E. coli* strains isolated from animals and later confirmed for human strains, it was verified that colibacilli may produce a low-molecular weight heat-stable toxin which can also cause, though more rapidly, a positive rabbit ileal loop test⁹. ST toxin as reported by DEAN et al.² is also able to evoke the same picture in the intestines of infant mice. Opposite to LT toxin, ST produces neither rounding of Y1 cells nor PF⁷.

With exception to Trabulsi's paper (1964¹⁰, São Paulo, Brazil) in which the occurrence of enterotoxigenic *E. coli* has been demonstrated and researches¹¹ presently being carried out at the Department of Microbiology and Parasitology of the "Escola Paulista de Medicina", SP, Brazil, nothing was found regarding other reports on the existence of ST toxin-producing strains of *E. coli* in Brazil, detected by DEAN's technique.

Due to those considerations and to the importance of the subject we think it is opportune to report the occurrence in Campinas, SP, Brazil, of diarrhea in children under three years old, whose stools revealed the presence of ST enterotoxin-producing strains of *Escherichia coli* at the bacteriological examinations.

MATERIAL AND METHODS

Forty-one strains of *Escherichia coli* isolated from diarrheal stools of adults and children were sent to us by the Central Laboratory of the Faculty of Medical Sciences, University of Campinas, SP, Brazil.

The microorganisms were isolated and biochemically classified as recommended by EDWARDS & EWING⁴, and serological classification, when carried out, was done by slide agglutination tests using OB antisera purchased from Wellcome Reagents Ltda. The following monovalent antisera were used: 026B6, 055B5, 0111B4, 0119B14, 0125B15, 0126B16, 0127B8, 0128B12, 086B7 and 0114K90.

Escherichia coli strains to be examined for enterotoxin production were first cultured in nutrient broth incubated at 37°C for 18 hours. Amounts of 25 ml of Evans medium⁶ distributed in 500-ml Erlenmeyer flasks were inoculated with 1 ml of nutrient-broth cultures. After incubation at 37°C in a rotatory incubator shaker and agitated at 135-150 rev/min cultures were spun down at 5000 rpm for 30 minutes. To supernatants carefully collected with a Pasteur's pipette, thimerosal at 1:10000 was added and the preparations so obtained then kept at -20°C till use.

As recommended by DEAN et al.², infant mice, aged 1-4 days, in number of four for each *E. coli* strain were injected with 0.1 ml of the ST-preparation obtained as described above. After four hours, the animals were sacrificed and the fluid accumulation response quantified by calculating the ratio of the combined weight of the intestine of the four mice to the combined weight of the bodies. An intestine to body weight ratio over 0.085 was regarded as indicative of enterotoxin production.

Strains which provided positive results in this test were inoculated in 1-liter Erlenmeyer flasks containing 100 ml of Evans' medium and incubated as described for ST-enterotoxin production. Supernatants of these cultures were afterwards 60x concentrated as described by EVANS et al.⁶ and injected in the rabbit depilated skin to verify if ST-producing strains elaborate also LT. Simultaneously, bacteria-free filtrates obtained as described for LT, without thimerosal, were tested in Y1 cells in order to establish whether there was production of any LT-toxin detected by this technique³.

RESULTS

Three out of 41 *Escherichia coli* strains caused an intestine to body weight ratio greater than 0.085.

The presence of a gut that appeared turgid, swollen and liquid filled was characteristic of a positive test allowing usually clear-cut identification of ST-producing strains, as shown in Fig. 1.

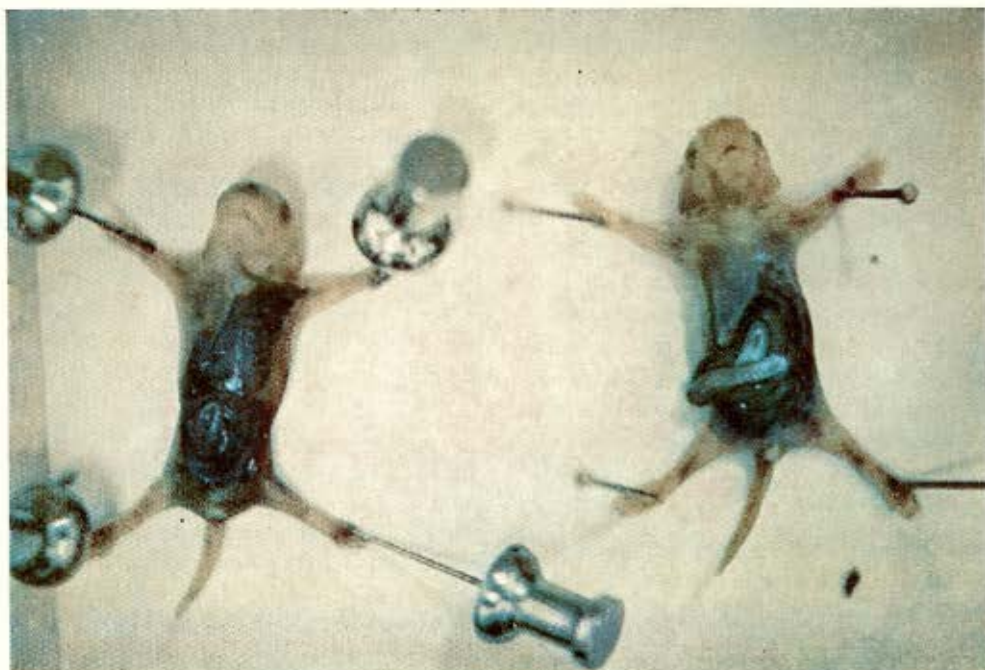


Fig. 1 — Left — Infant mouse inoculated with culture filtrate of a negative ST-producing *E. coli* strain. Right — Infant mouse inoculated with culture filtrate of *E. coli* strain 3, showing dilated intestines.

The strains 3, 54 and 55 produced in repeated tests means of 0.142, 0.123 and 0.122 respectively. The negative strains produced always values between 0.050-0.059 (21 strains) and 0.06-0.069 (15 strains) followed by two strains which gave values of 0.070 and 0.072.

Filtrates of ST-producing strains checked in Y1 cells did not cause any rounding. Concentrated (60x) preparations were unable to elicit an increase of local vascular permeability proving that the studied strains produced only ST toxin.

The serological classification performed by slide agglutination tests using OB antisera revealed that strains 3 and 55 belonged to serotype 0128B12. Strain 54 did not agglutinate with any antisera used.

DISCUSSION

Although the occurrence of *Escherichia coli* strains which give a positive ileal loop assay has been already reported among us in 1964¹⁰ information on the frequency and characterization of toxin produced are

still required.

Recently several strains producing concomitantly ST and LT toxins have been isolated in São Paulo, Brazil¹¹, but, in regard to the isolation of colibacilli producing only toxin of the ST type we have not found any reports in this country.

Considering that in the present work, among 41 strains, three were producing ST toxin, it might be suggested that this kind of toxin is more involved in the etiology of diarrheal diseases caused by *E. coli*. However it must be reminded that tests for LT production were not included in our routine work being carried out only with those strains which were confirmed to produce ST toxin.

Anyhow, it is apparent from the results — 3 (7.31%) positive ST-producing strains among 41 checked by the infant mouse test² — that the participation of these strains in infantile diarrhea may be important mainly if we agree that many cases of diarrhea from which colibacilli were isolated might be due actually to other causes.

Another relevant point is that contrarily to LT strains, which frequently are not included in classical enteropathogenic serotypes, two out of three ST-producing strains isolated by us belonged to serotype 0128B12.

Though relative to few strains the data obtained by us may indicate, as it has been pointed out by other Authors, that strains involved in infantile diarrhea and belonging to known recognized pathogenic serotypes when toxigenic produce preferentially if not exclusively ST toxin.

RESUMO

Isolamento de amostras de *Escherichia coli* produtoras de toxina termoestável de casos de diarréia em Campinas, SP, Brasil

Três amostras de *Escherichia coli* isoladas de casos de diarréia infantil na região de Campinas, SP, demonstraram produzir toxina do tipo ST detectada pelo teste de DEAN. Essas mesmas amostras, quando estudadas na linhagem Y1 de tumor de adrenal e na pele de coelhos, não produziram toxina do tipo LT. Em todos os casos as amostras produtoras de toxina ST forneceram no teste de DEAN relações entre o peso dos intestinos/peso do corpo iguais ou maiores que 0,122. Vinte e uma amostras não produtoras de ST deram relações entre 0,050 e 0,059; 15, entre 0,060 e 0,069 e apenas dois, entre 0,070 e 0,075.

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