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Four Biochemical Tests for Identification of Probable Enteroinvasive *Escherichia coli* Strains

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ABSTRACT. Enteroinvasive *Escherichia coli* (EIEC) share important features with *Shigella* spp., but EIEC strains are difficult to identify because their biochemical reactions are variable, and Sereny tests or other biological and molecular assays are expensive or hard to perform. The aim of this work was to detect probable enteroinvasive *E. coli* strains by using four biochemical tests, in children under 5 years of age with and without acute diarrhea. 330 strains of *E. coli* isolated from children with diarrhea, and 660 strains from children without diarrhea were studied. All strains were tested with the following tests: mucate, lysine and ornithine descarboxylase and motility. The strains which were negative to the four tests were tested by Sereny assay. Twelve strains (3.6%) isolated from children with diarrhea were negative to the tests proposed; eleven were lactose positive and only one was lactose negative. Three strains (0.5%) from children without diarrhea were negative to the tests proposed and were lactose positive. All the 15 strains (100%) were positive in Sereny assay. We recommend the use of these four biochemical tests for initial detection of EIEC strains, because their cost is very low and it is feasible carry out them in small diagnostic laboratories.

Key Words: Enteroinvasive *Escherichia coli* (EIEC), Identification

RESUMEN. *Escherichia coli* enteroinvasiva (EIEC) comparte importantes características con *Shigella* spp., pero las cepas EIEC son difíciles de identificar debido a que sus reacciones bioquímicas son variables a que la prueba de Sereny u otras ensayos biológicos y moleculares son caros o difíciles de realizar. El objetivo de este trabajo fue detectar probables cepas de *E. coli* enteroinvasivas mediante el uso de cuatro pruebas bioquímicas en niños menores de 5 años de edad con y sin diarrea aguda. Fueron estudiadas 330 y 660 cepas de *E. coli* aisladas de niños con y sin diarrea respectivamente. A todas las cepas se les hicieron las pruebas de mucato, lisina, ornitina descarboxilasa y movilidad. Las cepas que fueron negativas a las cuatro pruebas fueron sometidas al ensayo de Sereny. Veinte cepas (3.6%) aisladas de niños con diarrea fueron negativas a las pruebas propuestas; once fueron lactosa positivas y sólo una fue lactosa negativa. Tres cepas (0.5%) provenientes de niños sin diarrea fueron negativas a las pruebas propuestas y fueron lactosa positivas. Las 15 cepas (100%) fueron positivas en el ensayo de Sereny. Nosotros recomendamos el uso de estas cuatro pruebas bioquímicas para la detección inicial de cepas EIEC, debido a su bajo costo y a su facilidad de llevarlas a cabo en laboratorios de diagnóstico pequeños.

Palabras Clave: *Escherichia coli Enteroinvasiva* (EIEC), Identificación.

INTRODUCTION

DuPont et al (1971) described some strains of *Escherichia coli* that could cause an invasive, dysenteric form of diarrheal illness in volunteers. This group of strains has been called enteroinvasive *E. coli* (EIEC).[8]

Enteroinvasive *E. coli* (EIEC) share important features with *Shigella* spp. Both are able to produce the dysenteric syndrome characterized by severe abdominal pain and frequent stools containing blood and mucus. Both pathogens have the ability to invade epithelial cells, which is dependent in both on the presence of a 120-140 megadaltons plasmid.[7]

Although initially was accepted that EIEC strains had biochemical and serological similarity with *Shigella*,[3] posterior studies showed that EIEC strains are more difficult to identify because their biochemical reactions are variable, not all of them are of classical EIEC serotypes, and Sereny tests or tissue culture invasive assays are hard to perform with large numbers of isolates. In a study carried out in Thailand during 1985, 10% of EIEC strains identified were not among the classical serogroups and a new

serogroup, O171, was identified.[4] Studies carried out in several countries had shown frequencies of isolation of EIEC from 1 to 7%.[1,3,11]

One of the most characteristic features in the epidemiology of EIEC is the trend in age-specific isolation similar with *Shigella*; EIEC is not isolated from children under 1 year old with diarrhea and become prevalent in children among 3 to 5 years.[11]

Although *Shigella* species can be identified by standard bacteriologic methods, EIEC are more difficult to identify because their biochemical reactions are variable. Enteroinvasive *E. coli* strains were though lactose negative, but in several studies was observed that EIEC may be lactose negative or positive.[4,7,11] EIEC can be detected initially by biochemical and serological tests, but the diagnosis must be confirmed by biological assays: Sereny test, lethality in chicken embryo, invasiveness to epithelial cells, DNA probes, ELISA, PCR, etc.[10]

In a study performed by Echeverria et al. in Bangkok (1988), several EIEC serogroups were isolated. Analyzing the biochemical tests used in the study, they observed that EIEC were usually ornithine descarboxylase negative (87%), lysine descarboxylase negative (91%), nonmotile (91%), and mucate negative (100%).[4]

In Chile, Faundez et al. (1998) isolated 17 EIEC strains (1.9%) from 912 children with diarrhea and 3 EIEC strains (0.3%) from 1,112 children without diarrhea. All of them were Sereny positive, 5 did not belong to the classic EIEC serogroups, 19 (95%) were lysine descarboxylase negative, 17 were nonmotile (85%), 13 (65%) were lactose positive and 16 (80%) were ornithine descarboxylase negative.[7]

The aim of this work was to detect enteroinvasive *E. coli* strains by using four biochemical tests, in children under 5 years of age with and without acute diarrhea, in order to provide a preliminary and cheap test.

MATERIAL AND METHODS

330 strains of *E. coli* isolated from children under 5 years old with acute diarrhea, and 660 strains of *E. coli* isolated from children of the same age without diarrhea were studied. Isolation of *E. coli* strains were performed according to Edwards and Ewing, and biochemical identification according to the A.S.M.[6]

All *E. coli* strains were tested with the four following tests: 1, Mucate, mucate broth with bromothymol blue as indicator; 2, Lysine descarboxylase, lysine descarboxylase broth; 3, Ornithine descarboxylase and motility; 4, MIO medium (motility, indole and ornithine).

All the strains which were negative to the four tests were tested by Sereny assay.[9]

RESULTS

Twelve strains (3.6%) isolated from children with diarrhea were negative to the four biochemical tests proposed, 11 of them were lactose positive and only one was lactose negative.

Three strains (0.5%) isolated from children without diarrhea were negative to the four biochemical tests proposed; all of them were lactose positive.

All these 15 strains (100%) were positive in Sereny assay (Table No. 1).

DISCUSSION

In 1977, Beutin et al. in Germany, from 35 *E. coli* strains belonging to the EIEC-associated serogroups, only 11 strains were confirmed for DNA test and Sereny assay. All of them were lysine descarboxylase negative.[2]

Toledo and Trabulsi (1983), found an association in EIEC strains between the absence of lysine descarboxylase and Sereny test positivity; they observed that EIEC isolates were predominantly lactose negative.[13] Taylor et al. in Bangkok, Thailand (1988), isolated 23 EIEC strains, all of them were mucate negative, 21 were lysine descarboxylase negative and nonmotil, 20 strains were ornithine descarboxylase negative and 15 were lactose positive. All the strains were Sereny and DNA probes positive.[11]

Biochemical reactions of EIEC isolated from children with and without acute diarrhea in Merida, Yucatan, Mexico.

Biochemical reactions	Children with diarrhea 12/330	Children without diarrhea 3/660
Lactose negative	1/12	0/3
Lysine descarboxylase negative	12/12	3/3
Ornithine descarboxylase negative	12/12	3/3
Motility negative	12/12	3/3
Mucate negative	12/12	3/3
Sereny positive	12/12	3/3

In our work, we found EIEC strains in 3.6% children with diarrhea, and 0.45% children without diarrhea, which agree with the well-known epidemiology of these bacteria.[4,5,7,12]

All the strains that gave negative the four biochemical tests were positive to Sereny test, which confirmed their probable classification as EIEC.

On the other hand, from the 15 EIEC strains, 14 (93.3%) were lactose positive, and only one was lactose negative, which confirm that EIEC strains are not like *Shigella* (lactose negative). We recommend the use of these four biochemical tests for initial detection of EIEC strains, adding that their cost is very low, great experience is not required, and it is feasible carry out them in small diagnostic laboratories, and leave other tests like Sereny, DNA probes, etc., for confirmation in Reference Centers.

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