THE JOURNAL OF PREVENTIVE MEDICINE
2001; 9(3): 67-73

SETOTYPES OF *ESCHERICHIA COLI ENTEROINVASIVE* IN NORTHEASTERN DISTRICTS OF ROMANIA

Sofia Constantiniu[1], I.Buzdugan[2], Angela Romaniuc[1], L. Cozma[2], Raluca Filimon[1], Mariana Dumbrava[1], Anca Nistor[1], Lucia Teodoru[2], Paraschiva Onu[3], Gabriela Danis[2]

1. Institute of Public Health Iasi
2. Districtual Department of Public Health Vaslui
3. Medical Units Nicolina Iasi

Abstract. 30 *E.coli enteroinvasive* (EIEC) strains were studied concerning their biochemical characteristics, antigenic behaviour and the pathogenicity *in vivo* and *in vitro*. The strains were isolated from children with acute diarrhoea and patients of a foodborne outbreak . All strains presented the general biochemical reactions of the *Escherichia* genus and some characteristics resembling with *Shigella* genus – nonmotile, fermentation of glucose whithout gas production, negative for lysine decarboxilase.
According to slide and in tubes agglutination tests the strains were included in the following serotypes: O_{25} , O_{28}, O_{42} , O_{112} , O_{124} , O_{136} , O_{142} , O_{144}.
From 34 strains which agglutinated with anti – EIEC monovalent sera only 30 (85.7%) had the ability to produce keratoconjunctivitis on guinea pig's eye. On Congo red agar all EIEC strains were unpigmented. It is not a correlation between the capacity to bind the dye and the pathogenicity of EIEC strains.
Key-words: *E.coli*, enteroinvasive, biochemical characteristic, serotype, pathogenicity

Rezumat. 30 tulpini de *E.coli* enteroinvaziv au fost studiate din punct de vedere biochimic, antigenic şi provind patogenitatea pe ochi de cobai. Tulpinile au prezentat caracterele biochimice generale ale genului *Escherichia* şi unele caractere asemănătoare genului *Shigella* – imobilitate, fermentarea glucozei fără producere de gaz, absenţa lizin-decarboxilazei.Pe baza aglutinării pe lamă şi în tub, tulpinile au fost încadrate în serotipurile O_{25} , O_{28} , O_{42} , O_{112} , O_{124} , $O_{136,}$ O_{142} , $O_{144.}$ Din 34 tulpini care au aglutinat la tub cu serurile monovalente anti-EIEC numai 30 (85,7%) au produs keratoconjunctivita pe ochi de cobai. Pe agar cu roşu Congo tulpinile de EIEC au dezvoltat colonii de culoare roz, demonstrând incapacitatea de a lega colorantul.
Cuvinte cheie : *E.coli*, enteroinvaziv, caractere biochimice, serotipie, patogenitate

E.coli group is a common cause of diarrhea children in developing and some industrialized countries. ***E.coli* enteroinvasive (EIEC)** group that has the capacity to penetrate and multiply in epithelial cells, represents an important enteric pathogen in different area of the world. This agent causes mostly mild form of dysentery. Majority of EIEC strains have a virulence encoding plasmid of 120-140 mDaltons which is identical of those of *Shigella* group. All tests that can be applied for the determination of the virulence of *Shigella* species are suitable for testing EIEC

virulence: the Sereny test, HeLa invasivity test, detection of the presence of plasmid genes by DNA hibridization, the presence of plasmid – encoded outer membrane proteins by ELISA (1, 2, 3, 4).

The aim of our study was to summarize data concerning EIEC serotypes in north-eastern area of the country in the last five years and to determine biochemical characteristics and the pathogenicity of these strains.

MATERIAL AND METHODS

E.coli **enteroinvasive** isolations included:

- 25 *E.coli* strains from 1003 children with acute diarrhoea from health units. The samples were inoculated on Drigalski medium.10 colonies were selected from Drigalski medium and stored on nutrient agar slant.
- 5 *E.coli* strains isolated from hospitalized patients with acute diarrhoea in an epidemic focus of foodborne origin.

All *E.coli* isolates were investigated biochemicaly and by agglutination with anti EIEC I and II polyvalent sera supplied by Cantacuzino Institute Bucharest and with monovalent sera prepared in our laboratory with reference strains received from Statens Serum Institute Copenhagen. The strains were agglutinated on the slide and in tubes.

The pathogenicity of the strains was tested *in vitro* and *in vivo*. The *E.coli* strains agglutinable with specific monovalent sera were cultured on Congo red agar (Congo red Merck 0.01%) to determine their capacity to bind the dye.

The pathogenicity of the strains was tested *in vivo* by Sereny test – the capacity of inducing keratoconjunctivitis in the guinea pig's eye.

RESULTS AND DISCUSSION

EIEC group was isolated from 30 (2.4 %) children. All EIEC strains were confirmed by positive Sereny test.

EIEC strains shown the general biochemical reactions of the *Escherichia* genus and some similar characteristics with *Shigella* (nonmotile, the fermentation of glucose without gas production).

All strains revealed identical reactions in the following tests:

- positive for: catalase, acid production from glucose, manitol, production of indole, red methyl reaction;
- negative for: oxidase, urea, Simmons citrate, Voges – Proskauer, adonitol, inositol, salicin, dulcitol, cellobiose, gelatin – table 1.

Some biochemical reactions were intermediate area between *Shigella* and the classical characteristics of *Escherichia:* the motility, production of gas from glucose, lactose, lysine, ornithine, arginine, sacharose, salicin, raffinose, sorbitol, arabinose, rhamnose, xylose, thehalose, glycerol, maltose, esculin – table 1. It must point out that there are the strains agglutinable whith EIEC polyvalent and monovalent sera, non-pathogenic for guinea pig's eye, which had an intermediate biochemical behaviour between *Shigella* and *Escherichia*.

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Tab.1. Biochemical characteristics intermediate between *Escherichia* and *Shigella* of EIEC strains

Serotype	Motility	Glucose acid	Glucose gas	Lactose plate	Sacharose	Arabinose	Xylose	Trechalose	Maltose	Salicin	Raffinose	Rhamnose	Esculin	Sorbitol	Lysine	Ornithine
O_{25} n = 2	+	+	-	+	-	-	-	+	+	+	-	+	+	+	-	+
O_{28} n = 3	-	+	-	-	-	-	-	+	+	-	-	+	-	+		-
O_{42} n = 3	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-
O_{112} n = 8	-	+	-	+	-	-	-	+	+	+	+	+	+	+	-	-
O_{124} n = 4	+	+	+	+	-	+	-	+	-	+	-	-	+	+	-	-
O_{136} n = 3	+	+	+	+	-	+	-	+	-	+	+	-	+	+		-
O_{142} n = 3	+	+	+	+	-	-	+	-	+	-	-	+	-	-	+	-
O_{143} n = 2	-	+	+	+	-	+	-	+	+	-	-	+	-	+	-	-
O_{144} n = 2	-	+	-	-	-	+	-	+	+	+	-	+	+	+	+	+

Biochemical characteristics can not differentiate the pathogenic EIEC (KC^+) strains and *E.coli* strains agglutinable with polyvalent and monovalent sera but with negative Sereny test. This aspect was pointed out by Tonciu et al (5) in a study on *E.coli* strains which agglutinated with anti EIEC A or B polyvalent sera but don't produce keratoconjunctivitis in guinea pigs so cannot be considered as pathogenic EIEC strains.

254 *E.coli* strains agglutinable with I and II polyvalent sera were agglutinated with monovalent sera corresponding to the polyvalent serum (figure 1). Agglutination in tubes was performed only with those strains which agglutinated on slide with > +++ intensity.

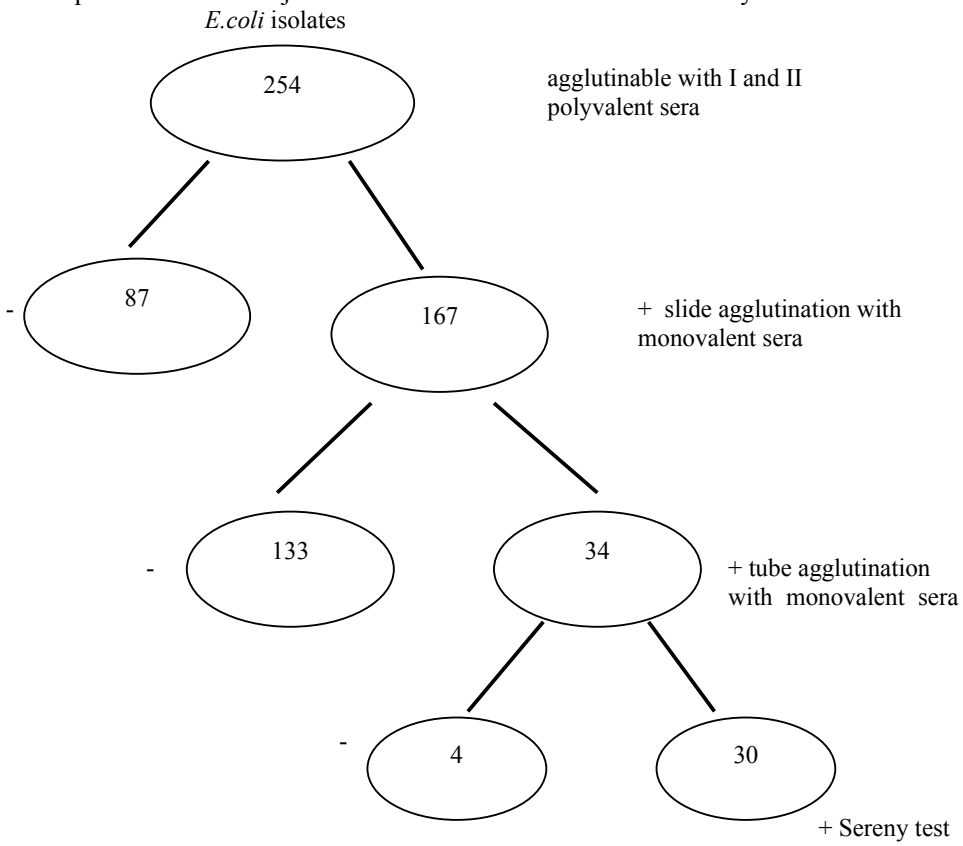

Figure no.1. Antigenic behaviour and pathogenicity of EIEC strains

The tube agglutination reactions of *E.coli* strains KC^+ and KC^- are presented in the table no. 2.

Tab. 2. Tube agglutination reactions of *E.coli* strains with anti-EIEC monovalent sera

Serum dilution / Monovalent serum	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	No.strains agglutinable on slide	No.strains positive*
O_{25}	5	5	4	1	**1**	**1**	0	5	2
O_{28}	6	2	2	2	2	**2**	**2**	6	3
O_{42}	4	44	4	4	3	**3**	0	5	3
O_{112}	8	8	8	8	8	**5**	**5**	12	8**
O_{124}	8	8	4	3	3	**3**	**3**	8	4
O_{136}	3	3	3	3	**3**	0	0	3	3
O_{142}	4	4	3	3	3	**3**	0	5	3
O_{143}	5	5	5	3	**2**	0	0	7	2
O_{144}	3	3	3	3	2	**2**	0	5	2

* strains agglutinated at 1/1 – 1/2 of serum's titer; ** 5 strains isolated from epidemic focus

From 167 strains which agglutinated on slide with monovalent sera only 34 (20.3%) agglutinated up to 1/1 titer or ½ of titer's serum as follows:

- one strain for O_{115} , O_{145} , O_{147} , O_{167} serogroupes, all these strains with negative Sereny test;
- two strains for O_{25} , O_{143}, O_{144} serogroups;
- three strains for O_{28} , O_{42} , O_{136} and O_{142} serogroups;
- four strains for O_{124} serogroup;
- eight strains for O_{112} serogroup.

The most frequently were isolated O_{124} and O_{112} serogroupes, the last serotype including and 5 strains isolated from a foodborne outbreak after fish 's consumption. The O_{25}, O_{28} , O_{42} , O_{136} , O_{142} and O_{144} were present in the similar proportion.

Our results confirm the circulation of many serogroups of EIEC in north-eastern part of the country. In 1988 we described an acute diarrhoeal disease episode with EIEC serotype O_{42} in a newborns department unit (6). In Romania it was also reported the most frequent serogroups O_{124} , O_{136} , O_{112} and O_{144} (5, 7). In another parts of the world the serogroups O_{28} ,O_{112} , O_{115} , O_{124} , O_{136} , O_{143} , O_{145} , O_{147} are the commonly incriminated (1, 2, 8).

30 (88.0%) out of 34 strains agglutinated in titers of the monovalent sera had the ability to produce keratoconjunctivitis on guinea pig's eye. 4 (11.7%) strains were serotyped but they had negative Sereny test. It was demonstrated by DNA probes for the invasiveness plasmid that EIEC strains possess a large plasmid of 120 – 140 mDaltons that encode all the genes necessary for the virulence and confer the ability to invade epithelial cells and producing an inflamatory diarrhoea similar to that cause *Shigella* species. (1-4). After 3 – 6 months all our EIEC KC^+ strains losed their capacity to produce positive Sereny test.

All EIEC strains formed on Congo red agar only pink colonies.In our experiments the *Shigella flexneri* 1b and 2a serotypes strains formed both pigmented and unpigmented colonies.

Our results indicate a poor relationship between the capacity to bind the dye and the pathogenicity of *Shigella* and EIEC strains, so don't confirm the possibility of use Congo red binding test as a screening for differentiation EIEC from the lots of *E.coli* serogroups.

CONCLUSIONS

- 1003 children with acute diarrhoeal disease were bacteriologically examined for the presence of *E. coli* enteroinvasive group;
- 25 EIEC strains isolated from sporadic cases and 5 EIEC strains from an epidemic focus were biochemical and antigenic tested;
- EIEC strains had some intermediate characteristics between *Shigella* and *Escherichia* (nonmotile, lysine – decarboxilase negative, fermentation of glucose without gas);
- EIEC strains were included in serogroups: O_{25}, O_{28} , O_{42} , O_{112} , O_{124} , O_{136}, O_{142} , O_{143} and O_{144};
- From 34 strains which agglutinated in 1/1 or ½ of monovalent sera's

titers only 30 had the positive Sereny test;

- All EIEC KC^+ were unpigmented on Congo red agar.

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