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Correlation Between Biochemical and Serological Characteristics of *Escherichia coli* and Results of the Serény Test

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A total of 1,335 *Escherichia coli* strains isolated from patients with diarrhea were submitted to the Serény test. Of these strains, 1,035 were also submitted to slide agglutination test, using antisera for invasive *E. coli* serogroups. Of 325 isolates that were lysine decarboxylase negative, 138 reacted with one of the *E. coli* antisera used. From this group of 138, 137 were positive in the Serény test. Strains that were lysine decarboxylase positive (1,010 strains) were all negative in the Serény test. These results show a close correlation between the biochemical and serological characteristics of *E. coli* and the results of the Serény test.

The term invasive *Escherichia coli* refers to certain *E. coli* strains that evoke experimental keratoconjunctivitis in the guinea pig (Serény test) and cause a *Shigella*-like disease in man. Although the known invasive *E. coli* strains belong to a restricted number of O serogroups and—most of the time—exhibit typical biochemical behavior (3), they are routinely identified by the Serény test (2), without any previous screening. The Serény test is easy to perform but, like most animal models for detection of bacterial virulence, it has the disadvantages of being expensive, troublesome, and potentially damaging as a source of infection for technicians and investigators. In view of the difficulties related to this test, invasive *E. coli* are not searched for by most laboratories.

The purpose of this paper is to present the results of a large number of Serény tests carried out with a wide variety of *E. coli* strains. The data obtained suggest that the outcome of the test can be fairly predicted from the biochemical and serological characteristics of the *E. coli* strains.

MATERIALS AND METHODS

E. coli strains isolated in routine stool cultures from children and adults with diarrhea were used. The strains were identified as *E. coli* on the basis of their behavior in the following biochemical tests: production of gas in glucose, production of H_2S, hydrolysis of urea, deamination of phenylalanine or tryptophan, decarboxylation of lysine, motility, and utilization of citrate in Simmons citrate agar. When necessary, additional tests were used. Initially, these tests were performed according to Edwards and Ewing (1), and deamination of tryptophan was not included. From 1977 on, phenylalanine was substituted by tryptophan, and all tests were carried out in a simplified system developed and evaluated in our laboratory (M. R. F. Toledo, C. F. Fontes, and L. R. Trabulsi, Rev. Microbiol., in press). Based on our previous experience (3, 5, 6), all lysine-negative *E. coli* strains (325 strains) were regularly tested in O antisera against the invasive *E. coli* serogroups already known. Each antiserum was introduced into the study as soon as the serogroup was described as invasive. In addition to these strains, 710 lysine-positive strains were tested in the same antisera. All of the strains referred to before (1,035 strains), as well as 300 other lysine-positive strains, totaling 1,335 strains, were submitted to the Serény test. Antisera were prepared with unheated and nonmotile cultures, according to the methods recommended by Edwards and Ewing (1) for the preparation of *E. coli* OK antisera. The following antisera were used in this study: O28ac, O29, O112ac, O124, O136, O143, O144, O152, O164, and "*E. coli*-São Paulo" (3, 5). As a routine technique, we used slide agglutination tests. However, all lysine-positive strains that strongly agglutinated in the slide tests were also tested in tubes, using heated suspensions (100°C, 1 h) of the cultures. All motile strains that agglutinated approximately in the titer of any O antisera had their H antigens determined by the methods recommended by Edwards and Ewing (1). During the period of the study, we used both lactose-positive and lactose-negative colonies growing on the usual media for isolation of *Shigella*. The Serény test was performed as described by its author (2).

RESULTS

All lysine-positive strains (1,010 strains) were negative in the Serény test. Among the 710 lysine-positive strains tested in the O antisera, a small number agglutinated to the titer of one of the antisera used. Among the lysine-negative motile strains, nine (10.5%) were Serény posi-

TABLE 1. Production of lysine decarboxylase, motility, agglutination in O antisera for invasive serogroups, and results of Serény test of 1,335 *E. coli* strains

No. of strains	Lysine	Motility	No. (%) of positive strains	
			O antisera[a]	Serény test
300	+	+	NT	0 (0)
520	+	+	10 (1.9)	0 (0)
190	+	−	4 (2.1)	0 (0)
86	−	+	9[b] (10.5)	9[b] (10.5)
239	−	−	129 (53.9)	128 (53.5)

[a] Not all strains were tested in the 10 antisera used in this study (see the text). NT, Not tested.
[b] All strains were O124:H30.

tive, but all isolates belonged to serotype O124:H30. Among the lysine-negative and nonmotile strains, 128 (53.5%) yielded a positive Serény test (Table 1). The results of the Serény tests with all lysine-positive and lysine-negative bioserotypes found in this study are shown in Table 2. With the exception of the single strain of bioserotype O164:H$^-$, all remaining lysine-negative and nonmotile bioserotype strains were Serény positive. It should also be noted (Table 2) that the nine motile Serény-positive strains belonged to serotype O124:H30. All lysine-positive bioserotypes were Serény negative, and, with the exception of serotypes O152:H$^-$ and O164:H$^-$, all were also motile. All bioserotypes shown in Table 2 yielded negative results in the tests for production of H_2S, urease, phenylalanine or tryptophan deaminase, and growth in Simmons citrate.

DISCUSSION

The results obtained show a very close correlation between the biochemical and serological characteristics of *E. coli* and the results of the Serény test. With one exception, all strains that were lysine negative and reacted strongly with one of the antisera in slide agglutination tests yielded a positive Serény test. On the other hand, Serény-negative tests were obtained with all lysine-positive strains, whether or not they reacted with the antisera used. The fact that some Serény-negative strains agglutinated to the titer of antisera O28ac, O29, O124, O143, O152, and O164 does not mean that invasive and noninvasive strains have identical O antigens. Several known and possible unknown O-antigen relationships may explain this finding, and only the use of cross-absorption tests may clarify the

TABLE 2. Correlation between *E. coli* bioserotypes and the Serény test

O:H type[a]	No. of strains	No. of strains positive in:					
		Lysine	Motility	Glucose (gas)	Indole	Lactose[b]	Serény test
O28ac:H$^-$	30	0	0	1	30	30	30
O28ac:H21, H25, H37, and H? (one of each)	4	4	4	4	4	4	0
O29:H$^-$	2	0	0	2	2	2	2
O29:H10, H21, and H? (one of each)	3	3	3	3	3	3	0
O124:H$^-$	8	0	0	7	8	0	8
O124:H30	9	0	9	8	9	0	9
O124:H19	1	1	1	1	1	0	0
O136:H$^-$	37	0	0	33	37	8	37
O143:H$^-$	5	0	0	4	5	5	5
O143:H4	1	1	1	1	1	1	0
O144:H$^-$	7	0	0	6	7	6	7
O152:H$^-$	35	0	0	32	35	3	35
O152:H$^-$	4	4	4	4	0	0	0
O152:H40	1	1	1	1	1	1	0
O164:H$^-$	1	0	0	1	1	1	0
NT:H$^-$ (*E. coli*-São Paulo)	4	0	0	4	4	4	4

[a] Symbols: H$^-$, nonmotile; H?, not tested; NT, negative with O1-O166 antisera (new O serogroup).
[b] Observed in isolation plates.

problem. On the other hand, it is also possible that within O serogroups, virulence is related to particular bioserotypes. Antisera for further studies of these noninvasive strains are in preparation in our laboratory.

The correlation found in this study indicates that invasive *E. coli* strains may be easily recognized by the use of a few biochemical tests and slide agglutination tests in antisera prepared appropriately. Among the biochemical tests, the most important one seems to be lysine decarboxylation. Motility is also very helpful, since motile invasive strains seem to occur only in serogroup O124. Furthermore, data in Table 1 indicate that about 50% of lysine- and motility-negative strains may be positive in the Serény test. In addition to the tests in Table 2, interested readers may find other useful tests for identification of invasive bioserotypes in a previous paper from our laboratory (3). The biochemical characteristics of several invasive bioserotypes have also been described by Stenzel (4).

Despite the high correlation between bioserotypes and invasiveness, it is too early to dispense with the Serény test to identify invasive *E. coli* strains routinely. More studies are necessary on the biochemical characteristics of a larger number of strains from different geographic areas and on the cross-reactions of the O antigens of the invasive bioserotypes. Furthermore, international agreement should be reached on the reagents and techniques to be used for the identification of these bacteria. However, we think that the procedure we are using at present in our laboratory can be recommended for the screening of *E. coli* strains for the Serény test: lactose-negative or -positive colonies growing on MacConkey, salmonella-shigella, or Hektoen plates (1) are submitted to the biochemical tests mentioned before. Those *E. coli* strains that do not decarboxylate lysine and are nonmotile are submitted to slide agglutination tests in polyvalent and monovalent antisera, prepared as described above. If the strain agglutinates in one of the polyvalent antisera, it is tested in the corresponding monovalent antisera. If agglutination occurs, we do the Serény test. If the strain is lysine negative but motile, the slide agglutination test is performed directly in O124 antiserum. In areas where no studies have been done on the biochemical behavior of invasive strains, one alternative procedure would be to screen in polyvalent antisera any *E. coli* strain, independently of its biochemical characteristics. Those that intend to use our procedure should keep in mind that probably we do not yet know all invasive *E. coli* serogroups that exist in nature.

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