Biochemical and Cultural Characteristics of Invasive *Escherichia coli*

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The biochemical characteristics of 97 invasive *Escherichia coli* strains of different O serogroups were studied. Considered as a group, the behavior of the strains was quite variable. However, none of them decarboxylated lysine and all but seven strains, belonging to the O124 serogroup, were nonmotile. The growth of 25 strains obtained on MacConkey, salmonella-shigella, xylose-lysine-desoxycholate, and Hektoen enteric agars was compared. MacConkey and Hektoen enteric agars yielded the highest average growth for these strains, whereas salmonella-shigella agar had the lowest average counts.

Invasive *Escherichia coli* strains are enteropathogenic for humans and yield a positive result in the Serény test (8, 12, 20, 22). In that respect they are similar to the *Shigella* group. Invasive *E. coli* strains have been described in the following O serogroups: O28a,c (20); O29 (18); O42 (24); O112a,c (13); O124 (9); O136 (3, 19); O143 (24); O144 (2); O152 (14, 15, 21); and O164 (7, 10). Recently, a new invasive *E. coli* strain (*E. coli* São Paulo), probably having an undescribed O antigen, was isolated in our laboratory (M. R. Toledo, M. H. L. Reis, and L. R. Trabulsi, submitted for publication).

Very little is known about the biochemical behavior of these bacteria as a group. However, some studies, carried out with individual serogroups, have shown that they have intermediary properties between those presented by *Shigella* and *E. coli* (10, 11, 16, 20). In the present study, we report the biochemical characteristics, and the growth capacity on selective media, of invasive *E. coli* strains belonging to all but two serogroups (O42 and O112a,c) described.

MATERIALS AND METHODS

Bacterial strains. A total of 97 strains belonging to the following O serogroups were studied: O28a,c (14 strains), O29 (2 strains), O124 (12 strains), O136 (31 strains), O143 (2 strains), O144 (2 strains), O152 (30 strains), and O164 (1 strain). Three strains of *E. coli* São Paulo mentioned before were also included. Eighty strains were isolated in our laboratory during the last 15 years, the majority of them from the feces of patients with diarrhea. Three strains (*E. coli* O124/3192-75, *E. coli* O143/3363-64, and *E. coli* O144/5385-72) were kindly provided by Betty R. Davis, Center for Disease Control, Atlanta, Ga., and the 14 remaining strains were sent to us for identification. All of the strains were identified by the usual biochemical and serological procedures (1, 19, 20) and caused a severe keratoconjunctivitis in guinea pigs (Serény test).

Biochemical tests. The biochemical reactions of the 97 strains were carried out by using the methods recommended by Edwards and Ewing (1); however, negative fermentation tests were observed for only 2 weeks. The β -galactosidase test was performed by the method of Lowe (6). Utilization of L-rhamnose, D-xylose, or L-serine as a sole source of carbon, each source of which was tested with 76 strains, was investigated by using the base of Simmons medium for citrate utilization (1). The final concentration of each substance was 0.2%; the media were inoculated and incubated, and the results were interpreted in the same way as with Simmons citrate agar (1).

Growth on selective media. To evaluate the growth capacity of the strains, dilutions of broth cultures of 25 strains in logarithmic-phase growth were plated on MacConkey (MC) (Difco Laboratories), salmonella-shigella (SS) (Difco), xylose-lysine-desoxycholate (XLD), prepared as specified by Taylor (17), Hektoen (HE) (Oxoid Ltd.), and heart infusion (HIA) (Oxoid Ltd.) agars. After 18 h of incubation at 37°C, the number of colonies on each medium was recorded and the percentage of growth was calculated considering the growth obtained on HIA as 100%.

RESULTS

The biochemical characteristics of 97 invasive *E. coli* strains, according to the serogroup, are shown in Table 1. It should be noted that none of the strains decarboxylated lysine and all were nonmotile, with the exception of seven strains in O124 serogroup which were motile. All the motile strains had the flagellar antigen H30. In the other tests used to distinguish *Shigella* from *E. coli* such as utilization of sodium acetate, mucate, and citrate in Christensen medium, gas production from glucose, and lactose fermentation, the behavior of the strains was widely variable. A summary of the biochemical characteristics of all the strains is shown in Table 2.

The results of the tests for utilization of L-

TABLE 1. Biochemical reactions of 97 invasive *E. coli* strains of different *O* serogroups

Test	No. of positive strains ^a								<i>E. coli</i> São Paulo
	O28a,c	O29	O124	O136	O143	O144	O152	O164	
Indole (Kovacs)	14	2	12	31	2	2	30	1	3
Methyl red	14	2	12	31	2	2	30	1	3
Voges-Proskauer	0	0	0	0	0	0	0	0	0
Christensen citrate	0	(1)	1 (1)	1 (7)	0	0	6 (10)	(1)	(2)
Simmons citrate	0	0	0	0	0	0	0	0	0
Sodium acetate	0	(1)	9 (3)	8 (8)	1	1	24 (4)	0	3
Growth in KCN	0	0	0	0	0	0	0	0	0
H ₂ S (TSI) ^b	0	0	0	0	0	0	0	0	0
Christensen urea	0	0	0	0	0	0	0	0	0
Malonate	0	0	0	0	0	0	0	0	0
Mucate	0	0	7	0	2	0	30	0	1
Jordan tartrate	0	0	9	0	1	0	1	0	1
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0
Arginine dihydrolase	0	0	(3)	0	(1)	0	4 (1)	0	(3)
Lysine decarboxylase	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase	9 (4)	2	0	28 (2)	0	(2)	0	0	0
Motility	0	0	7	0	0	0	0	0	0
β -Galactosidase (ONPG) ^c	14	2	12	18	2	2	30	0	3
Glucose (acid)	14	2	12	31	2	2	30	1	3
Glucose (gas)	0	2	10	26	2	1	27	0	3
Lactose	13 (1)	2	(11)	7 (1)	2	1 (1)	2 (27)	0	3
Mannitol	14	2	12	31	2	2	30	1	3
Sucrose	0	0	0	1	(1)	1 (1)	0	0	(3)
Salicin	0	0	0	2 (6)	0	0	18 (6)	0	0
Dulcitol	0	0	9 (3)	0	0	0	26 (2)	0	0
Inositol	0	0	0	0	0	0	0	0	0
Adonitol	0	0	0	0	0	0	0	0	0
Raffinose	0	0	0	0	2	2	0	0	3
Sorbitol	13 (1)	1	12	31	1 (1)	0	28 (2)	1	3
Arabinose	14	2	12	31	2	2	30	1	3
Rhamnose	14	2	(7)	30 (1)	2	2	2 (20)	1	3
Xylose	14	(2)	12	31	2	2	29 (1)	1	3
Trehalose	14	2	12	31	2	2	30	1	3
Esculin	0	0	0	1 (5)	(1)	0	7 (15)	0	(2)
Glycerol (acid)	10 (4)	2	9 (3)	31	1 (1)	2	29 (1)	1	3
Glycerol (gas)	0	2	7 (1)	22 (4)	1	(1)	25 (1)	0	3
Cellobiose	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0
Maltose	2 (7)	2	12	25 (4)	(1)	2	23 (7)	1	1 (2)
Melibiose	11	2	10	29 (1)	2	1	25 (2)	1	3
Total of strains	14	2	12	31	2	2	30	1	3

^a Number in parentheses indicates positive reaction after 3 or more days of incubation.^b TSI, Triple sugar iron agar.^c ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

rhamnose, L-serine, and D-xylose, individually, as the sole carbon source (Table 3) show that L-rhamnose was utilized by only 12 strains (15.8%), whereas L-serine and D-xylose were utilized by 65 (85.5%) and 68 (89.5%) strains, respectively.

The growth of 25 strains of different serogroups on MC, SS, XLD, and HE media shows that the less inhibitory media were MC and HE (Table 4). The SS medium was remarkably inhibitory for strains of some serogroups.

DISCUSSION

Considered as a group, invasive *E. coli* strains are rather heterogeneous in their biochemical characteristics. Nevertheless, the presence of an invasive strain can be suspected by the results of some tests. The lysine decarboxylase test seems to be the most important among them. All the 97 strains yielded negative results in this test (Table 1), and it is well established that 89.5% of *E. coli* strains are lysine decarboxylase

TABLE 2. Summary of the biochemical reactions of 97 invasive *E. coli* strains^a

Test	Reaction	% Positive	(% Positive)
Indole (Kovacs)	+	100.0	
Methyl red	+	100.0	
Voges-Proskauer	—	0	
Christensen citrate	d	8.3	(22.7)
Simmons citrate	—	0	
Sodium acetate	d	47.4	(16.5)
Growth in KCN	—	0	
H ₂ S (TSI) ^b	—	0	
Christensen urea	—	0	
Malonate	—	0	
Mucate	— or +	41.2	
Jordan tartrate	— or +	12.4	
Phenylalanine deaminase	—	0	
Arginine dihydrolase	d	4.1	(8.3)
Lysine decarboxylase	—	0	
Ornithine decarboxylase	d	40.2	(8.3)
Motility	—	7.2	
β -Galactosidase (ONPG) ^c	+ or —	85.6	
Glucose (acid)	+	100.0	
Glucose (gas)	+ or —	73.2	
Lactose	d	30.9	(42.3)
Mannitol	+	100.0	
Sucrose	—	2.1	(5.2)
Salicin	d	20.6	(12.4)
Dulcitol	d	36.1	(5.2)
Inositol	—	0	
Adonitol	—	0	
Raffinose	—	7.2	
Sorbitol	+	92.8	(4.1)
Arabinose	+	100.0	
Rhamnose	d	57.7	(28.9)
Xylose	+	96.9	(3.1)
Trehalose	+	100.0	
Esculin	d	8.3	(23.7)
Glycerol (acid)	+	90.7	(9.3)
Glycerol (gas)	d	61.9	(7.2)
Cellobiose	—	0	
Erythritol	—	0	
Maltose	d	70.1	(21.7)
Melibiose	d	86.6	(3.1)

^a Figures and signs in parentheses indicate positive reaction after 3 or more days of incubation. Symbols: +, 90% or more positive; —, 90% or more negative; + or —, majority of strains positive, occasional strains negative; — or +, majority of strains negative, occasional strains positive; d, different reactions (+, [+], —).

^b TSI, Triple sugar iron agar.

^c ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

positive (1). Also, the motility test can be helpful since it was positive only for seven strains belonging to the O124 serogroup, which represents 7.2% of the total number of strains (Tables 1 and 2). These findings suggest that lysine-positive or typical motile *E. coli* strains should not be considered as invasive strains and, therefore, not be submitted to the Séreny test for invasiveness.

A more reliable suspicion of the presence of

TABLE 3. Utilization of L-rhamnose, L-serine, and D-xylose as a sole source of carbon

O sero-group	No. of strains	No. of positive strains ^a		
		L-Rham-nose	L-Ser-ine	D-Xyl-ose
O28a,c	9	3 (1)	7	5 (2)
O29	1	0	1	0
O124	12	0	11	12
O136	24	4 (1)	22	19 (3)
O143	1	0	0	(1)
O144	2	1	0	1
O152	26	(2)	24	23 (1)
O164	1	0	0	(1)
Total	76	8 (4)	65	60 (8)

^a Number in parentheses indicates positive reaction after 3 or more days of incubation.

TABLE 4. Growth of invasive *E. coli* strains on selective media

O sero-group	No. of strains	Media (% of growth) ^a			
		MC	SS	XLD	HE
O28a,c	5	56.7	31.4	74.3	75.9
O29	1	100.0	100.0	79.1	100.0
O124	5	73.0	43.3	55.0	70.5
O136	5	77.9	63.3	59.8	85.9
O143	1	100.0	27.8	83.6	81.6
O144	2	97.6	0	60.6	88.7
O152	5	71.9	84.4	87.6	86.9
O164	1	100.0	95.5	91.1	84.9

^a Percent was evaluated considering the growth on HIA as 100%. Averages are as follows: MC, 84.6%; SS, 55.7%; XLD, 73.9%; HE, 84.3%.

an invasive *E. coli* strain is gained by considering, at the same time, the serogroup and the results of some biochemical tests such as lactose fermentation, mucate and sodium acetate utilization, and some others, in addition to the lysine decarboxylase and motility tests.

Some serogroups present a typical pattern of biochemical activity as can be seen in Table 1. All of the O28a,c strains ferment lactose, do not produce gas in glucose, and are sodium acetate negative; none of the O124 and O152 strains decarboxylate ornithine, and most of them ferment lactose slowly. It must also be noted that the majority of invasive *E. coli* strains are Jordan tartrate negative, except O124, whereas *E. coli* is generally positive (99.5%). In the same way, mucate is fermented by 93.0% of *E. coli* strains (1) but is not fermented by the majority of invasive *E. coli* strains, with the exception of O124 and O152.

The results of the tests for utilization of L-rhamnose, L-serine, and D-xylose as sole source of carbon (Table 3) suggest that these tests are not helpful for the identification of invasive *E.*

coli. The only exception would be the test for utilization of L-rhamnose which yielded negative results for most of the strains. According to Véron and Le Minor (23), about 95% of *Shigella* strains are unable to utilize these three substrates as a sole source of carbon, whereas most *E. coli* strains can. However, it should be mentioned that the base medium used by these authors was not the same that we used.

The results of the usual biochemical tests are very similar to those observed by other authors who studied some of the serogroups included in this paper (10, 11, 16, 24). However, they do not support the concept of some investigators on the accuracy of invasive *E. coli* identification by its ability to decarboxylate lysine, ferment mucate, and utilize sodium acetate as a sole carbon source (4).

Data in Table 4 show that invasive *E. coli* strains should not be looked for only on MC, a medium often used for isolation of *E. coli*. In fact, the growth of many serogroups was even better on HE, a medium which has been found to yield good results in the isolation of *Shigella* and *Salmonella* (5). Some serogroups demonstrated good growth on SS agar, so invasive *E. coli* strains might also be isolated on this medium.

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