INFECTION AND IMMUNITY, Nov. 1978, p. 334-338
0019-9567/78/0022-0334$02.00/0
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Vol. 22, No. 2
Printed in U.S.A.

Prevalence of Enterotoxigenicity in Human and Nonhuman Isolates of *Yersinia enterocolitica*

CHIK H. PAI,[1]* VERA MORS,[1] AND S. TOMA[2]

Department of Microbiology, McGill University-Montreal Children's Hospital Research Institute, Montreal, Quebec[1]; and National Reference Service for Yersinia, Public Health Laboratory, Ontario Ministry of Health, Toronto, and Laboratory Centre for Disease Control, Ottawa, Ontario,[2] Canada

Received for publication 23 May 1978

A total of 414 cultures of *Yersinia enterocolitica* isolated from human and nonhuman sources were examined for heat-stable enterotoxin (ST) production to determine whether enterotoxigenicity was related to the source of isolation, serotype, or biochemical characteristics of the organism. A total of 65% of all cultures were found to produce ST. Enterotoxin production was much more prevalent in strains isolated from humans (218/232) than in those isolated from animals (17/34), water (9/49), raw milk (14/44), and food (10/55). Strains belonging to the serotypes O:3; 8; 5,27; 6,30; 9, often isolated from human infections, were almost always enterotoxigenic (191/196), although ST production was also highly prevalent among a few other serotypes. The most significant difference was observed between the groups that differed in the ability to ferment rhamnose; only 13 of 130 rhamnose-positive isolates produced ST (10%) compared to 255 of 284 rhamnose-negative cultures (90%). These results suggest that ST production is ubiquitous in *Y. enterocolitica*, with the highest prevalence among strains associated with human infections.

Yersinia enterocolitica infection in humans has been recognized with increasing frequency in recent years (21). Diverse clinical manifestations have been reported (33), but children most frequently have acute gastroenteritis (10, 17; L. Lafleur, C. Pai, O. Hammerberg, and G. Delage, Third International Symposium on *Yersinia*, Mont Gabriel, Quebec, Canada, 1977). *Y. enterocolitica* is the second commonest cause of bacterial gastroenteritis in children in the Montreal area: from June 1977 to March 1978, 169 *Salmonella*, 130 *Y. enterocolitica*, and 36 *Shigella* were isolated from children with diarrhea at the Montreal Children's Hospital and St. Justine Hospital (M. I. Marks, C. H. Pai, L. Lafleur, O. Hammerberg, L. Lackman, and L. Chicoine, Pediatr. Res., abstr. no. 794, 12:496, 1978).

Y. enterocolitica has been isolated frequently from wild and domestic animals (11, 15, 23, 29, 30), water (14, 18, 29; G. P. Jansen and T. N. Saari, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Q68, p. 272), raw milk (25), and a variety of food sources (13). The significance of the common water and food isolates is unclear, as they tend to be different in their antigenic structure and biochemical reactions from those frequently associated with human illness. There has been only one documented foodborne outbreak of *Y. enterocolitica* (3), although food sources were implicated in other outbreaks (1, 35).

Lee et al. (19) found that typical human isolates invaded HeLa cells while none of the food and water isolates tested were invasive. Une et al. (31) reported that only those "human" serotypes, O:3; 5,27; 8 and 9, were able to penetrate HeLa cells, suggesting that epithelial cell infectivity in vitro could be used for the determination of pathogenicity of *Y. enterocolitica*.

In an earlier study we found all of 43 isolates of serotype O:3 to produce heat-stable enterotoxin (ST), while only 2 of 10 nontypable strains tested were enterotoxigenic (22). This enterotoxin was found to be similar to *Escherichia coli* ST in a number of characteristics; it was stable at 100°C for 15 min and active in the infant mouse and rabbit ileal loop systems, but not in the Y1 adrenal cell assay. However, further investigation is required to establish their identity. The present study was undertaken to determine whether there are differences in enterotoxin production of human and nonhuman isolates and to see whether a correlation would exist between the enterotoxigenicity and the serotype, biotype, or biochemical characteristics of *Y. enterocolitica*. (This paper was presented in part at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 14 to 19 May 1978).

MATERIALS AND METHODS

Bacterial strains. A total of 414 cultures of *Y.*

enterocolitica (232 of human and 182 of nonhuman origin) were examined; their sources and serotypes are shown in Table 1. A total of 135 strains were isolated from stool specimens from children with diarrhea and their household contacts presented to The Montreal Children's Hospital or St. Justine Hospital in Montreal. The remaining 279 strains were from the stock cultures maintained at National Reference Service for *Yersinia*, Toronto. Serotyping was done by slide agglutination, using 34 absorbed and unabsorbed O-antisera (32) prepared in rabbits. Biotyping was performed by the method of Wauters (G. Wauters, thesis, Université de Cathol. de Louvain, Louvain, Belgium, 1970; for Wauter's biotype schema, see reference 27).

Enterotoxin assay. ST activity was assayed in the infant mouse system (9) by using sterile culture filtrates obtained from 48-h cultures incubated on a rotary shaker (200 rpm) at room temperature (22). Each sample was tested in a group of two to three mice, and results were interpreted by visual examination of the intestine. When the intestine was dilated with visible accumulation of fluid, the test was interpreted as positive. If the result was not clear, the intestine and the remaining body were weighed to calculate the ratio of intestine to remaining body weight. A ratio of 0.09 or greater was taken as positive, and that of 0.08 or less was taken as negative. Tests were repeated when the ratio was between 0.08 and 0.09.

Heat-labile enterotoxin (LT) activity was assayed in the Y1 mouse adrenal cell system (24) by using the culture filtrates as described above. Strains of *E. coli* positive for LT and/or ST were used as controls in both assays.

RESULTS

Of 414 isolates of *Y. enterocolitica*, 268 (64.7%) were positive for ST. None produced LT.

Serotype. Of 159 strains belonging to serotypes O:3, 8, and 9, most commonly associated with human infections (2, 3, 21, 30, 34), 158 were enterotoxigenic (Table 2). Enterotoxigenicity was as prevalent among strains of serotypes O:5,27 and O:6,30, the second and third most frequent isolates from humans in Canada (30). The majority of serotypes O:5; 6,31; 7,8; 13,7 and 16 were also ST positive. These serotypes of human origin have been reported (7; S. Toma, L. Lafleur, and V. Deidrick. Third International Symposium on *Yersinia*, Mont Gabriel, Quebec, Canada, 1977). ST production was infrequent among the strains belonging to O:1,2,3 (1/4), O:4,33 (0/5), O:14 (0/5), O:17 (0/11), and O:18 (0/5). Only 18.9% of nontypable strains were found to be enterotoxigenic.

Source of isolation. A total of 94% of human, 50% of animal, and 22% of environmental isolates were found to produce ST (Table 3). For a given serotype, the source of isolation was not found to be the determining factor for enterotoxigenicity (Table 4). Of 232 human isolates, 183 were derived from stool, 36 from nonenteric, and 13 from unknown sources. Enterotoxigenicity was as prevalent among the nonenteric isolates as in the stool isolates.

TABLE 1. *Serotype and source of isolation of Y. enterocolitica culture studied*

Serotype (O:)	No. of strains isolated from:				
	Humans	Animals	Water	Raw milk	Food
1		3			
1, 2, 3	1		3		
3	130	1			
4	5			1	
4, 32	4	1			2
4, 33	1	1		2	1
5	6			2	1
5, 27	15	3			
6, 30	11		3	3	2
6, 31	4	3	1	2	
7, 8	4			1	
8	21	2			1
8, 19	1				
9	3	1			
10					1
11		2			1
11, 24			2		
12					2
13, 7	3	1		2	
14			2	2	1
15				1	1
16	5		2		
16, 29	1				
17	2	1	5	1	2
18			2	1	2
21	4	1	3	1	
34	2	2	3		1
NT[a]	9	12	23	25	37

[a] NT, Nontypable.

TABLE 2. *Relationship of serotypes to enterotoxigenicity of Y. enterocolitica*

Serotype (O:)	No. tested	ST positive	
		No.	%[b]
3	131	130	99.2
8	24	24	100.0
9	4	4	100.0
5, 27	18	17	94.4
6, 30	19	16	84.2
5	9	9	100.0
6, 31	10	9	90.0
7, 8	5	5	100.0
13, 7	6	5	83.3
16	7	5	71.4
Other types	75	24	32.0
NT[a]	106	20	18.9

[a] NT, Nontypable.
[b] Average percentage was 64.7.

Biotype. *Y. enterocolitica* cultures were distributed among four biotypes with 90.5% belonging to biotypes 1 and 4 (Table 5). A high (>84%) incidence of ST production was evenly distributed among all four biotypes in the case of human isolates, with a much lower (<45%) incidence distributed among biotypes of nonhuman isolates. No association could be made between enterotoxin production and the biotypes.

Rhamnose fermentation. Because rhamnose-positive strains of *Y. enterocolitica* are considered atypical (6, 8), ST production was compared in cultures that differed in rhamnose fermentation (Table 6). A large majority (89.8%) of rhamnose-negative isolates were enterotoxigenic, whereas only 10% of rhamnose-positive cultures produced ST. A low prevalence of enterotoxigenicity among nonhuman isolates (Table 3) could be explained by the fact that about 2/3 of these isolates were rhamnose positive. Among nonhuman isolates, ST production was much more frequent in the rhamnose-negative group.

Salicin and esculin reactions. Knapp and Thal (16) divided *Y. enterocolitica* into typical and atypical groups on the basis of a number of biochemical reactions including salicin and esculin hydrolysis: salicin- and/or esculin-positive strains were considered atypical. Furthermore, Lee et al. (19) reported an association of HeLa cell invasiveness with the clinical isolates that were salicin and esculin negative. In the present study, salicin- and esculin-negative strains were found to produce ST much more frequently (187/193, or 96.9%) than did strains that were salicin and/or esculin positive (66/197, or 33.5%). However, the majority of the strains belonging to the latter group were also rhamnose positive. Therefore, enterotoxigenicity was compared among rhamnose-negative strains that differed in salicin and esculin reactions. The difference observed between these two groups was no longer significant when rhamnose-positive strains were excluded: 54 of 72 salicin/esculin-positive and 186 of 191 salicin/esculin-negative cultures were enterotoxigenic.

TABLE 3. *Relationship of source of isolation to enterotoxigenicity of Y. enterocolitica*

Source of isolation	No. tested	ST positive	
		No.	%
Humans	232	218	94.0
Animals	34	17	50.0
Water	49	9	18.4
Raw milk	44	14	31.8
Food	55	10	18.2

TABLE 4. *Association of enterotoxigenicity with serotypes regardless of source of isolation*

Serotypes (O:)	Human isolates		Nonhuman isolates	
	No. tested	No. ST positive	No. tested	No. ST positive
5	6	6	3	3
6, 30	11	11	8	5
6, 31	4	4	6	5

TABLE 5. *Relationship of biotype to enterotoxigenicity of Y. enterocolitica*

Biotype	Human isolates		Nonhuman isolates		Total (%)
	No. tested	No. ST positive	No. tested	No. ST positive	
1	81	71	162	42	46.5
2	19	16	9	4	71.4
3	1	1	10	3	36.4
4	130	129	1	1	99.2

TABLE 6. *Relationship of rhamnose fermentation to enterotoxigenicity of Y. enterocolitica*

Rhamnose fermentation	Human isolates		Nonhuman isolates		Total (%)
	No. tested	No. ST positive	No. tested	No. ST positive	
Negative	218	213	66	42	89.8
Positive	14	5	116	8	10.0

DISCUSSION

The prevalence of ST production was examined with respect to five variables of *Y. enterocolitica* isolates; source of isolation, serotypes, biotypes, rhamnose fermentation, and salicin and esculin reactions. Enterotoxigenicity was found to be more frequent among the strains of (i) human origin, (ii) serotypes commonly associated with human infections, and (iii) those that were rhamnose negative. However, none of these variables are completely independent of others, and, therefore, no single characteristic could be used in distinguishing ST-positive from ST-negative strains. Nevertheless, rhamnose fermentation was found to be most significantly associated with enterotoxigenicity; 90% of rhamnose-negative isolates were found to produce ST compared to only 10% of rhamnose-positive strains (Table 6). The serotypes associated with human infections, O:3; 8, and 9, are always rhamnose negative. Rhamnose-positive strains are usually isolated from water, raw milk, and other food sources (13-15, 18, 25) and have been considered

atypical or called *Y. enterocolitica*-like on the basis of their biochemical reactions, cultural characteristics, serological properties, and DNA homology studies (6, 8). It has been suggested that rhamnose-positive strains be distinguished from typical strains and reported as a *Y. enterocolitica*-like rhamnose-positive group (5). ST production may be one more of the characteristics that are common to a large majority of typical *Y. enterocolitica* strains.

The significance of ST production in vitro by *Y. enterocolitica* is not clear at the present time. Specific surface antigens of colonization factors have been found to be a prerequisite for enterotoxigenic *E. coli* to cause diarrheal diseases in animals and humans (12, 26). The relationship between the O-antigens of *Y. enterocolitica* and their ability to adhere to the intestinal mucosa has not been established.

Pathological studies of some of the fatal cases of *Y. enterocolitica* infections have demonstrated an invasive pattern of enteritis with gross ulcerative lesions and histopathological features, including necrosis and marked inflammatory exudates (4). Similar findings have been reported from experimental infections in mice (7) and monkeys (20). Invasiveness of *Y. enterocolitica* has been demonstrated in HeLa cell systems (19, 31) and in guinea pig eyes (28). These observations, coupled with the previous findings from this laboratory that ST production by *Y. enterocolitica* could be demonstrated only when cultures had been grown at 30°C or below (22), are not consistent with a view that the enterotoxin of *Y. enterocolitica* plays an essential role in the pathogenesis of gastroenteritis in humans. An animal model of diarrhea is required to elucidate the role of *Y. enterocolitica* enterotoxin.

ACKNOWLEDGMENTS

We thank Simon Sorger and Velma Deidrick for their assistance in identification and collection of *Y. enterocolitica* isolates. The generous gift of *Y. enterocolitica* strains by Lucette Lafleur is greatly appreciated.

This investigation was supported by grant A9433 from the National Research Council of Canada and by a grant from the McGill University-Montreal Children's Hospital Research Institute.

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