JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1977, p. 341-345

Vol. 5, No. 3
Printed in U.S.A.

Yersinia enterocolitica: Recovery and Characterization of Two Unusual Isolates from a Case of Acute Enteritis

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Received for publication 7 October 1976

Enteritis caused by *Yersinia enterocolitica* appears to be an uncommon occurrence in the United States. Most of the reported cases have been caused by biochemically typical *Y. enterocolitica* serotype O:8, the most frequently encountered serotype in the United States. The present report describes the isolation of two biochemically and serologically unusual *Y. enterocolitica* isolates from a patient with acute enteritis. One strain was distinguished by the rapid fermentation of rhamnose and raffinose and by citrate utilization at 22°C but not at 37°C. The other isolate was sucrose negative, and at either temperature it lacked both the fermentative capability for rhamnose and raffinose and the ability to utilize sodium citrate. Neither strain was agglutinable with known *Y. enterocolitica* antisera. The rhamnose-positive isolate showed an increased resistance to ampicillin, cephalothin, colymycin, and penicillin when tested at 22°C as compared to results obtained at 37°C. The demonstration that one patient's serum contained agglutinins (1:64) against the sucrose-negative strain supports its etiological significance. The role of the rhamnose-positive strain in the patient's illness is speculative. It conceivably could have potentiated the pathogenicity of the sucrose-negative isolate.

Documented enteric infections due to *Yersinia enterocolitica* are still uncommon in the United States. During the period of 1939 through 1957, *Y. enterocolitica* was recovered from the feces of 10 symptomatic patients and recorded in the annual reports of the Division of Laboratories and Research of the New York State Department of Health (8-10, 22, 22a, 23). During the period of 1966 through 1972, after improvement in microbiological techniques for the identification of *Y. enterocolitica*, 4 of 29 isolates studied by Weaver and Jordan (28) at the Center for Disease Control were recovered from stool. In 1973, Gutman and colleagues (13) reported an interfamilial outbreak of enteritis in which *Y. enterocolitica* was recovered from the spleen of one patient at autopsy. Although this species was not recovered from the stools of 16 symptomatic patients involved in the outbreak, diagnostic titers of 1:512 or greater were observed in the sera of 8 of these patients. In 1975 Jacobs (14) and Greenwood and his colleagues (12), respectively, reported the isolation of *Y. enterocolitica* from the feces of a 6-month-old girl and a 26-year-old female with diarrhea. More recently, Bissett (4) reported on 24 strains of *Y. enterocolitica* which had been submitted to the California Department of Health during an 8-year period. Four of the 12 strains that had been recovered from feces were from patients with gastrointestinal symptoms. In the above cited instances, most of the isolates were biochemically identical to typical *Y. enterocolitica*, usually of serotype O:8, the most frequently encountered serotype in the United States (4, 28). Enteritis caused by biochemically or serologically "atypical" *Y. enterocolitica* is an infrequent occurrence in the United States. Equally rare is the recovery of two strains of *Y. enterocolitica* from the same fecal sample. To date, such reports have only appeared in the European (1) and Japanese (29) literature. In 1974 Bottone and his colleagues (5) reported on the recovery of 12 *Y. enterocolitica* isolates that differed from the more typical isolates because of their ability to ferment rhamnose and raffinose and to utilize sodium citrate at 22°C. Eight of these strains were of O:17. Three of the isolates were recovered from stool specimens; however, no clinical information was available.

The present communication describes the isolation of two biochemically and serologically unusual *Y. enterocolitica* isolates from a patient with acute enteritis. One of the isolates fermented rhamnose, raffinose, and sucrose and utilized sodium citrate, whereas the other isolate lacked these capabilities. Neither strain was agglutinable with known *Y. enterocolitica* antisera.

MATERIALS AND METHODS

Case report. E.O., a 23-year-old female, was seen in the emergency room of The Mount Sinai Hospital on 25 April 1976, with a chief complaint of crampy abdominal pain and nonbloody, nonmucoid diarrhea of 5 days' duration which was accompanied by a low-grade fever of 99.2°F (ca. 37.3°C). Two weeks before her present visit, the patient had experienced a similar episode which lasted several days. Stool guaiac in the emergency room was recorded as 1+. On physical examination, the abdominal cavity was soft and slightly tender below the umbilicus. There was no organomegaly, and the remainder of the physical examination was negative. Laboratory data included: a leukocyte count of 4,200 cm^3, with 50% polymorphonuclear leukocytes, 11% band forms, 35% lymphocytes, 3% eosinophiles, and 1% monocytes; 35% hematocrit; hemoglobin, 11.1 g/100 ml; and a sedimentation rate of 17 mm/h. The patient was sent home without treatment.

A rectal swab placed into gram-negative broth was submitted to the microbiology laboratory and streaked onto phenylethyl alcohol, endo, and hektoen-enteric (H-E) agars. After a 24- to 36-h incubation, numerous colorless colonies developed on the enteric media and, upon isolation and definitive characterization, were shown to be two distinct *Y. enterocolitica* strains.

With these available data, the patient was called back for reexamination on 28 April 1976. She was asymptomatic, but further questioning revealed that the patient worked at a Day Care Center where both the director and a teacher had had diarrhea prior to her first episode. The patient's 7-year-old sister developed diarrhea of "a few" days duration after a visit during her present illness. There was no history of animal contact. A blood sample was collected for serological studies.

A fresh fecal specimen was obtained on 29 April 1976. The new specimen was cultured as above, and, in addition, a 2- to 3-g quantity was added to a tube containing 5 ml of phosphate-buffered saline (pH 7.2) for cold enrichment as outlined by Paterson and Cook (20).

RESULTS

The first indication that *Yersinia* might be present in the original stool culture was a characteristic odor that accompanied the growth on endo and H-E agars after a 24-h incubation. The odor was that of "potato or cabbage," which was highly reminiscent of that commented upon by Wauters (27) and had also been noted with a previous isolation of a sucrose-negative *Y. enterocolitica* in our laboratory. Because of this suspicion, several colorless colonies were selected from the agar substrates and subcultured onto endo agar for purification. After a 24-hr incubation on the basis of the characteristic odor and subtle tinctorial differences in colony morphology, two separate non-lactose-fermenting, gram-negative organisms which were subjected to biochemical characterization at both 22 and 37°C incubation temperatures were observed.

Biochemical studies revealed two distinct *Y. enterocolitica* isolates (Table 1). One strain was distinguished by the rapid fermentation of rhamnose and raffinose and citrate utilization at 22°C but not 37°C, and fermentation of sucrose, melibiose, and xylose at both temperatures. The other isolate which had the marked

TABLE 1. *Temperature-related characteristics of the rhamnose-positive and sucrose-negative isolates of Y. enterocolitica*[a]

Test	*Y. enterocolitica*			
	Rhamnose-positive isolate at:		Sucrose-negative isolate at:	
	37°C	22°C	37°C	22°C
	Alk/acid	Alk/acid	Alk/acid	Alk/acid
KIA				
Citrate	−	+	−	−
Urease	+	+[w]	+	+[w]
Decarboxylase				
Ornithine	+	+	+	+
Lysine	−	−	−	−
Arginine dihydrolase	−	−	−	−
β-Galactosidase	+	+	+	+
Catalase	+	+	+	+
DNase	−	−	−	−
Phenylalanine deaminase	−	−	−	−
Indole	+	+	+[w]	+
Nitrate	+	+	+	+
Acid				
Melibiose	+	+	−	−
Salicin	+	+	−	−
Sucrose	+	+	−	−
Raffinose	−	+	−	−
Rhamnose	−	+	−	−
Xylose	+	+	−	−
Adonitol	−	−	−	−
Arabinose	+	+	+	+
Dextrose	+	+	+	+
Lactose	−	−	−	−
Maltose	+	+	+	+
Mannitol	+	+	+	+
Sorbitol	+	+	+	+
Trehalose	+	+	+	+
Bile esculin	+[w]	+	+	+
Motility	−	+	−	+
Growth on:				
H-E agar	−	−	+	+
EMB agar	+	+	+	+
Endo agar	+	+	+	+
MacConkey agar	+	+	+	+
Salmonella-shigella agar	−	−	−	−
Xylose-lysine-deoxycholate agar	+	+	+	+

[a] Symbols: +, Positive; −, negative; w, weak; *, results recorded after a 24-h incubation.

odor differed from typical *Y. enterocolitica* by being sucrose negative and was distinguished from the first isolate because it lacked a fermentative capability at either temperature for rhamnose, raffinose, melibiose, and xylose and in the ability to utilize sodium citrate. The isolates also differed in their capability to initiate growth on H-E agar. Only the sucrose-negative strain developed on this medium. The rhamnose-positive isolate failed to grow on this agar substrate at 22 or 37°C, which was consistent with previous observations (5). After a 24-h incubation at 22 and 37°C and subculture to eosin methylene blue (EMB), xylose-lysine-deoxycholate, and MacConkey agars, both strains produced pin-point colonies which increased in diameter from 0.1 to 0.5 mm after 48-h incubation. The growth of the rhamnose-positive isolate on EMB produced a green metallic sheen which imparted to the colonies a characteristic that is highly suggestive of fecal streptococci and *Escherichia coli* on this medium. Neither isolate grew on salmonella-shigella agar nor produced hemolysis on blood agar. Among the positive characteristics shared by the isolates were: motility at 22°C but not at 37°C; production of indole, β-galactosidase, urease, and ornithine decarboxylase activities; and fermentation of glucose without gas. Neither strain utilized lactose nor decarboxylated lysine or produced arginine dihydrolase.

Broth dilution susceptibility tests which had been performed at 22 and 37°C according to the method of Schneierson and Amsterdam (24) revealed an expanded temperature-dependent susceptibility pattern for the rhamnose-positive isolate, an observation that had been previously commented upon by Chester and Stotzky (7). This strain showed increased resistance to ampicillin, cephalothin, colymycin, and penicillin when tested at 22°C as compared to results obtained at 37°C. This phenomenon was not observed with carbenicillin, chloramphenicol, tetracycline, gentamicin, kanamycin, and tobramycin, antibiotics to which the isolate was susceptible. The sucrose-negative isolate displayed a temperature-dependent susceptibility only to chloramphenicol, being susceptible at 37°C and moderately resistant at 22°C. The remainder of the results were identical at both incubation temperatures, namely susceptibility to gentamicin, kanamycin, and tobramycin, moderate resistance to carbenicillin, and overt resistance to ampicillin, cephalothin, colymycin, penicillin, and tetracycline (Table 2).

Both isolates were forwarded to H. H. Mollaret (Pasteur Institute [Paris]) who confirmed their identity and performed the serological studies that showed them to be inagglutinable with a battery of known *Y. enterocolitica* antisera.

TABLE 2. *Antibiotic susceptibility patterns of rhamnose-positive and sucrose-negative isolates of Y. enterocolitica as determined at 37 and 22°C*

Antibiotic	Concn (μg/ml)[b]	*Y. enterocolitica*[a]			
		Rhamnose-positive isolate at:		Sucrose-negative isolate at:	
		37°C	22°C	37°C	22°C
Ampicillin	5, 10	S	MR	R	R
Cephalothin	7.5, 15	MR	R	R	R
Chloramphenicol	7.5, 15	S	S	S	MR
Colymycin	2, 5	S	R	R	R
Penicillin	1, 10 U	MR	R	R	R
Carbenicillin	25, 100	S	S	MR	MR
Gentamicin	5, 10	S	S	S	S
Kanamycin	10, 20	S	S	S	S
Tetracycline	2, 4	S	S	R	R
Tobramycin	5, 10	S	S	S	S

[a] As determined by the two tube broth dilution method (cf. 24).

[b] Abbreviations: R, Resistant; MR, moderate resistant; S, Sensitive.

The single serum sample which had been collected from the patient was tested for antibody to the two *Yersinia* isolates. An agglutinating titer of 1:64 was obtained against the sucrose-negative isolate. Antibody was not detected against the rhamnose-positive strain nor against *Y. enterocolitica* O:3, O:8, O:9, or O:17 or to several representative *Enterobacteriaciae* such as *E. coli, Klebsiella pneumoniae, Proteus vulgaris, Providencia* sp., or *Salmonella typhimurium*. Ten randomly selected sera which had been submitted to the serology laboratory failed to agglutinate either isolate.

Direct culture as well as cold enrichment of the second stool specimen at 4°C with subsequent subculture for periods up to 28 days failed to yield either *Y. enterocolitica* strain.

DISCUSSION

There are several factors that often interfere with the isolation and accurate identification of *Y. enterocolitica* from clinical specimens. In most diagnostic microbiology laboratories, stool specimens from patients with gastroenteritis are usually processed for the exclusive isolation of *Salmonella* and *Shigella*, in spite of the fact that enteritis due to *Y. enterocolitica* is a common occurrence (3, 11, 17), particularly in children (3, 11, 19, 25). The recovery of *Y. enterocolitica* from specimens in which it is the sole isolate, i.e., blood or mesenteric lymph nodes, does not present a particular problem. Its isolation, however, from fecal specimens that contain a multiplicity of species requires an appreciation of the diversity of its cultural profile on

commonly employed "enteric" media. Additionally, because of biochemical similarities to other *Enterobacteriaceae*, once isolated, the accurate identification of *Y. enterocolitica* may be hampered, especially when the isolated strain(s) differs in several respects from typical *Y. enterocolitica*.

The present report highlights some of the above difficulties. Culturally, the rhamnose-positive isolate did not grow on H-E agar being recovered only from endo agar. On EMB agar, the growth of this species was accompanied by a green metallic sheen similar to *E. coli* or fecal streptococci. The sucrose-negative isolate, on the other hand, was recovered from both endo and H-E agars and produced colorless colonies on EMB. Although neither strain grew on salmonella-shigella agar, most *Y. enterocolitica* isolates belonging to O:3, O:8, and O:9, the most frequently encountered serotypes in human infections, do develop on this medium. Additionally, typical strains of *Y. enterocolitica* are capable of growth on xylose-lysine-deoxycholate or H-E agar and ferment the sucrose and xylose in the former medium and salicin and sucrose in the latter, thereby rendering colonies indistinguishable from "coliforms" (26). In view of this metabolic and cultural heterogenicity, it is not at all unlikely that *Y. enterocolitica* is often not isolated or identified when present in stool. Therefore, it is difficult to accurately assess the true incidence of enteric yersiniosis due to typical *Y. enterocolitica*, and virtually impossible to gauge the incidence of infections due to biochemical variants which, as shown, may also vary in their growth patterns on enteric media.

Strains of *Y. enterocolitica* that are rhamnose positive or sucrose negative have only been infrequently encountered in humans (2, 4a). Although to date we have recovered 18 such isolates from human sources, rhamnose-positive isolates have been mainly recovered from environmental sources such as drinking water (16; T. H. Saari and T. J. Quan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C119, p. 45), cold-water trout (15), salt water shellfish, and ice cream (W. H. Lee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, P16, p. 202). These isolates have been mainly of O:14, O:16, or O:17 or were nontypable (2). Sucrose-negative isolates are mainly encountered from animal sources (H. H. Mollaret, personal communication) and seldom from humans. Weaver (28), Wauters (27), and Niléhn (18) have each reported one strain in their surveys, Bissett has reported two (4; one of these isolates was also reported by Weaver), and one strain was previously isolated in our laboratory. With the exception of the latter isolate, a O:12 (H. H. Mollaret, personal communication), the isolates of Weaver, Wauters, and Bissett were of O:11.

The exact taxonomic status of rhamnose-positive strains as well as sucrose-negative isolates is still unresolved. Brenner and colleagues (6) studied biochemically typical and atypical isolates of *Y. enterocolitica*, which included both rhamnose-positive and sucrose-negative strains. On the basis of deoxyribonucleic acid hybridization studies, these investigators distinguished three deoxyribonucleic acid-related groups of *Y. enterocolitica* and suggested a fourth. One group corresponded to biochemically typical *Y. enterocolitica* (irrespective of indole production); the second was comprised of rhamnose-positive, but raffinose- and melibiose-negative isolates; the third, rhamnose-, raffinose-, and melibiose-fermenting strains; the fourth, sucrose-negative isolates. Although these authors specified that only the first group is *Y. enterocolitica*, they favored the retention of all four groups within the genus *Yersinia*.

The recovery of a rhamnose-positive as well as a sucrose-negative strain from the same fecal specimen is a rather unique occurrence. Since neither of these strains was agglutinable with presently available *Y. enterocolitica* antisera, they may even represent new serotypes and are, hence, even more unusual. The question of the virulence of biochemically atypical strains of *Y. enterocolitica* is of fundamental importance, particularly when the isolate is recovered from a site containing other microbial species. *Y. enterocolitica* of serotypes other than O:3, O:8, and O:9 have been recovered from blood and other normally sterile sources (4, 28) in which their etiological significance is unquestioned. In the present report, the demonstration that the patient's serum contained agglutinins (1:64) against the sucrose-negative strain supports its etiological significance as the cause of the patient's gastroenteritis. Furthermore, one of the sucrose-negative isolates reported by Bissett (4) was also recovered from the feces of a patient who had experienced abdominal pain and diarrhea.

One can only speculate on the role of the rhamnose-positive isolate in the patient's illness. Although serum agglutinins were not demonstrated against this isolate, its possible significance cannot be eliminated on this basis alone. Ahvonen (1) has clearly shown that, unlike infections due to *Y. enterocolitica* O:3 and O:9, serum agglutinins are not found in every case of yersiniosis. None of four patients, from whom *Y. enterocolitica* O:6, O:7, and O:13 was recovered from the stool during

an illness that was suggestive of acute appendicitis, had detectable serum agglutinins against these isolates. Although it may be argued that the presence of the rhamnose-positive strain was coincidental rather than causal, it is also conceivable that it could have potentiated the pathogenicity of the sucrose-negative isolate. The answers to such questions regarding unusual *Y. enterocolitica* isolates depends on both clinicians and microbiologists having a heightened awareness to the possible presence of this microorganism.

ACKNOWLEDGMENTS

We acknowledge the technical skills of Joan Heitner and Victor Trason.

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