

Atypical *Yersinia enterocolitica*: Clinical and Epidemiological Parameters

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Infections due to biochemically typical *Yersinia enterocolitica* usually present as gastroenteritis, mesenteric lymphadenitis, terminal ileitis, and septicemia often with visceral abscesses. In these instances, the isolates have been biochemically typical and of well-established serotypes, namely 0:3 or 0:9 and, in the United States, 0:5 or 0:8. The recovery, recognition, and significance of biochemically and serologically atypical *Y. enterocolitica* in human infections has proceeded more slowly. From an analysis of the clinical histories of 20 patients infected with 21 such aberrant *Y. enterocolitica*, it appears that these strains are of restricted pathogenic potential, producing various clinical entities such as localized skin abscesses, conjunctivitis, self-limiting enteritis, and wound and urinary tract infections in hosts with predisposing factors. Epidemiologically, whereas episodic acquisition of atypical strains by hospitalized patients is indicative of nosocomial transmission, in the present series sporadic isolations over a 4-year period, mainly from ambulatory patients, suggest an occult reservoir in the community serviced by The Mount Sinai Hospital. In contrast to typical *Y. enterocolitica*, which has become well adapted in animal and human hosts, it appears that environmental strains may be in the evolutionary process of becoming adapted to humans.

Renewed interest in the United States in infections caused by *Yersinia enterocolitica*, a gram-negative coccobacillus of the family *Enterobacteriaceae*, has brought a flurry of reports incriminating this microorganism in gastroenteritis (20), mesenteric lymphadenitis (11), terminal ileitis (29), and bacteremia (21, 35). Outbreaks of infection within a family unit (17) and the more widespread outbreak of gastroenteritis and mesenteric lymphadenitis at Holland Patent, N.Y., involving 222 children (4) have further kindled the interest in this apparently heretofore overlooked bacterial pathogen. In these instances, the isolates have all been biochemically typical and of well-established, human infection-causing serotypes (i.e., serotypes 0:5 and 0:8), and the clinical presentations have been consistent with infection caused by this species.

Reports of the isolation of *Y. enterocolitica* strains of unusual biochemical profile and serotype, mainly from environmental (9, 38) and human sources (6, 7), have begun to be interspersed with the documentation of the more typical isolates from human infections. What remains unknown, however, is the capability of these aberrant strains to produce the more serious syndromes (such as mesenteric lymphadenitis and septicemia) analogous to those accompanying infection due to typical *Y. enterocolitica*.

During a 4-year interval (from April 1972 through April 1976), 21 isolates of biochemically atypical and serologically unusual *Y. enterocolitica* strains were recovered from 20 symptomatic subjects who presented a variety of clinical manifestations. Nineteen of these isolates fermented rhamnose, raffinose, and melibiose and utilized sodium citrate at 22°C. The microbiology of 12 of these isolates was previously described (6, 13). The two remaining isolates differed from typical *Y. enterocolitica* by their failure to ferment sucrose and to produce acetylmethylcarbinol. These isolations prompted the examination of the clinical histories of these patients to assess the pathogenic potential of these strains and to place into perspective their relationship to typical *Y. enterocolitica*.

MATERIALS AND METHODS

Specimens for culture were plated onto Trypticase soy agar with 5% sheep blood (BBL), Endo agar, and, for stool specimens, Hektoen-Enteric (H-E) agar in addition. A duplicate blood agar was inoculated with wound specimens and incubated anaerobically (GasPak, BBL). Isolates were characterized biochemically at 22 and 37°C as outlined by Edwards and Ewing (14). Serum samples obtained from several patients were tested for agglutinins to the isolated strain by the standard tube dilution procedure. Serological and bacteriophage typing of the various isolates was performed through the courtesy of H. M. Mollaret,

Pasteur Institute, Paris, and S. Toma of the Public Health Laboratories, Toronto, Ontario.

RESULTS

Clinical evaluation. Clinical significance was ascribed to the *Yersinia* isolates in those instances wherein it was recovered (i) from an extraintestinal site in pure culture or in combination with one other bacterial species or (ii) from a stool culture of a patient with abdominal pain or diarrhea, which failed to yield more frequently encountered bacterial pathogens such as *Salmonella* and *Shigella* species. In three of the four latter instances, analysis of a concomitant stool specimen for intestinal parasites was negative. Viral agents were not sought. No significance was attached to those *Yersinia* isolates obtained in conjunction with several other microbial species or when the isolate was not causally related to the patient's illness. Brief clinical histories are presented for 3 representative patients of the 13 in whom the *Yersinia* isolate was deemed significant. Patients 2 and 20 have been described in greater detail elsewhere (7, 16). Table 1 shows the source and pertinent data relating to the isolates.

Patient histories. Patient 1: urinary tract infection. This 43-year-old female patient had a 26-year history of idiopathic bladder neck obstruction and urethral stenosis with frequent episodes of dysuria. Usually, on these occasions, the urine contained numerous polymorphonuclear leukocytes and various microbial species. On admission, the patient was catheterized and 800 ml of urine was withdrawn. Bacteriological examination of a sample revealed 10^6 organisms per ml, later identified as a rhamnose-positive, serotype 0:17 *Y. enterocolitica*.

Patient 15: abscess. This 20-year-old woman had a 1.6-cm, fluctuant, painful, superficial, right axillary abscess of 4 days duration, which was surgically drained. No other history was elicited. Culture of the purulent exudate under aerobic and anaerobic conditions grew a rhamnose-positive, serotype 0:17 *Y. enterocolitica* and *Staphylococcus epidermidis*.

Patient 19: conjunctivitis. This 14-month-old female was seen 2 days previously for diarrhea productive of four loose stools daily. Vomiting was absent. On her present hospital visit, the child was febrile (101°F) and had bilateral conjunctivitis for which she was treated with gantrisin ophthalmic solution. Culture of the conjunctiva prior to therapy grew a sucrose-negative, serotype 0:12 *Y. enterocolitica* and *Enterobacter cloacae*.

Bacteriological characteristics. Each of the 21 isolates had the biochemical features of *Y. enterocolitica*: fermentative metabolism, pro-

duction of urease, ornithine decarboxylase, and B-galactosidase, motility at 22°C but not at 37°C, and lack of lysine decarboxylase, arginine dihydrolase, phenylalanine deaminase, and oxidase activities.

The rhamnose-fermenting isolates differed from typical *Y. enterocolitica* in their wider spectrum of temperature-dependent features (5, 12, 13). Utilization of sodium citrate and prompt fermentation of rhamnose, raffinose, and melibiose at 22°C, but weakly or not at all at 37°C, were noteworthy. These strains conformed most closely to Nilehn's biotype 1 (31). The two sucrose-negative isolates were identical to typical *Y. enterocolitica* except for their failure to ferment this carbohydrate and to produce acetyl-methylcarbinol at 22°C. According to Wauters (37), these strains are sufficiently different from Nilehn's five biotypes to warrant separate biotype status. None of the 21 isolates was phage typable (phage group 10) (30, 31).

The temperature-related antibiograms for 13 of the rhamnose-fermenting (12, 13) and the sucrose-negative (7) isolates have been reported. Essentially, most of the isolates were resistant to ampicillin, carbenicillin, cephalothin, and penicillin, and uniformly susceptible to chloramphenicol, gentamicin, kanamycin, tobramycin, and the combination of 1 µg of trimethoprim and 19 µg of sulfamethoxazole per ml.

DISCUSSION

In the United States in the early 1960s, Wetzler and Hubbert (38) reported the isolation of three rhamnose-positive strains of *Y. enterocolitica* from the feces of healthy deer. Subsequently, Botzler et al. (8) expanded their environmental studies and showed the presence of rhamnose-fermenting *Y. enterocolitica* in frogs and snails. Three of the eight isolates recovered from these sources also fermented raffinose and melibiose and utilized sodium citrate, and hence were similar to our isolates. Since these early descriptions, rhamnose-positive *Y. enterocolitica* have also been isolated predominantly from water (9, 19, 23, 36; T. H. Saari and T. J. Quan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C119, p. 45), animal (36), and food sources (18; W. H. Lee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, P16, p. 202).

The recovery, recognition, and significance, however, of rhamnose-fermenting *Y. enterocolitica* from human sources has proceeded at a much slower pace. The first report of such isolations appeared in 1974 (6). Since this report from our laboratory, seven additional strains have been isolated. Other human isolates in the United States included one encountered by Bissett (3), a serotype 0:16 sputum isolate. The

TABLE 1. *Clinical and laboratory data relevant to the 21 atypical Y. enterocolitica isolates*

Date	Patient no.	Sex	Age	Source	Biotype ^a	Serotype ^b	Associated organism(s)	Comment ^c
8-72	1	F	42 yr	Urine	1	0:17	None	Urethral stenosis
9-72	2	F	39 yr	Wound	1	0:17	<i>Staphylococcus aureus</i>	Postoperative infection
9-72	3	M	68 yr	Eye	1	0:17	<i>Staphylococcus epidermidis</i>	Bilateral conjunctivitis
9-72	4	F	40 yr	Eye	1	0:17	None	Chronic chalazion, right upper lid
10-72	5	M	6 yr	Stool	1	0:17	"No enteric pathogens"	Enteritis
10-72	6	M	Newborn	Eye	1	0:17	<i>Staphylococcus aureus</i>	Conjunctivitis
10-72	7	M	79 yr	Urinary catheter	1	0:17	<i>Aeromonas</i> sp. <i>Candida</i> sp. <i>Enterobacter</i> sp. <i>Streptococcus faecalis</i>	Splenectomy; colonic neoplasm
5-73	8	M	16 yr	Throat	1	NAG	None	Prophylactic erythromycin for RHD
5-73	9	M	Newborn	Eye	1	0:16	None	Bilateral conjunctivitis, URI
10-73	10	M	2 yr	Stool	1	NAG	"No enteric pathogens"	Enteritis
11-73	11	F	10 yr	Urine	2	0:17	<i>Acinetobacter</i> sp.	Count 75,000 organisms per ml; recurrent UTI
11-73	12	F	9 mo	Stool	1	NAG	"No enteric pathogens"	Frequent diarrhea for 3 mo; watery, non-bloody stool
6-74	13	M	3 yr	Cervical lymph node	1	NAG	None	Hodgkins
6-74	14	F	14 yr	Eye	1	NAG	<i>Pseudomonas</i> sp.	Conjunctivitis
9-74	15	F	22 yr	Axillary abscess	1	0:17	<i>Staphylococcus epidermidis</i>	Abscess of 4 days duration
7-75	16	M	22 yr	Eye	1	NAG	<i>Staphylococcus epidermidis</i>	Conjunctivitis 1 week after contact lens placement
7-75	17	F	6 yr	Eye	1	NAG	<i>Staphylococcus epidermidis</i> <i>Proteus rettgeri</i>	Inflammation of left eye for 2 days
8-75	18	M	9 yr	Knee fluid	1	0:17	<i>Staphylococcus aureus</i>	Suppurative arthritis
12-75	19	F	14 mo	Eye	?	0:12 ⁺	<i>Enterobacter cloacae</i>	Conjunctivitis
4-76	20	F	20 yr	Stool	?	NAG ⁺	"No enteric pathogens"	Enteritis
					1	NAG		

^a According to Nilehn (31).

^b NAG, Nonagglutinable with existing *Y. enterocolitica* antisera. +, Sucrose and rhamnose negative.

^c RHD, Rheumatic heart disease; URI, upper respiratory infection; UTI, urinary tract infection.

latter strain, recovered from a patient with abdominal pain, diarrhea, and fever, differed from those encountered in this laboratory by its failure to ferment raffinose or melibiose or to utilize sodium citrate at 22°C.

In the present series, of the 19 rhamnose-fermenting *Y. enterocolitica*, clinical significance could be ascribed to 11 isolates (patients 1-5, 9, 10, 12, 14-16), was questionable in 4 instances

(patients 8, 11, 18, 20), and was absent in 4 cases (patients 6, 7, 13, 17). The two sucrose-negative isolates (patients 19 and 20) were both considered significant.

The frequency of recovery of rhamnose-fermenting *Y. enterocolitica* from stool cultures of normal subjects is apparently rare (2, 27). In patients with enteritis but "no enteric pathogens," the incidence of rhamnose-fermenting

strains is unknown. Based upon our observations, it appears that their pathogenic potential is restricted, as judged by the presentation of a self-limiting enteritis without ensuing mesenteric lymphadenitis or terminal ileitis. Serum agglutinins may not develop in response to infection, as evidenced by the lack of anti-yersinia agglutinins in the sera of three patients (no. 5, 10, 20) from whose stool culture a rhamnose-positive strain was recovered. With patient 20, from whom a sucrose-negative strain was additionally isolated, serum agglutinins (1:64) were present only against this strain and not to the rhamnose-fermenting isolate.

Although the absence of agglutinins in some cases of yersiniosis is not uncommon (1), lack of antibody production to rhamnose-positive strains may be related to their apparent lack of invasiveness. Lee and colleagues (25) have shown that while typical clinical isolates of *Y. enterocolitica* attach, invade, and multiply within Hela cells, none of seven (including three stool isolates) of our rhamnose-fermenting clinical strains, tested by Lee and associates, possessed this capability. Based upon this observation, these investigators concluded that since rhamnose-fermenting strains were incapable of invasion in a system devoid of defense mechanisms, they "are most probably not invasive in animals or humans also." These seven strains, however, were not tested for enterotoxin production, which, if present, could have accounted for the gastrointestinal symptoms noted with our patients.

Infection with rhamnose-fermenting *Y. enterocolitica* also occurred in patients with predisposing factors. In one instance the isolate was recovered in pure culture from a urine specimen of patient 1, who had a history of chronic urinary retention accompanied by repeated episodes of bacterial cystitis. In patient 2, a rhamnose-utilizing *Yersinia* was present along with *S. aureus* in an undermining wound infection with sinus tract formation which developed postoperatively. Serum agglutinins to the yersinial isolate were absent. Since such a progressive infection is apparently uncommon for rhamnose-positive strains, in such settings these yersiniae may be regarded as "opportunistic pathogens."

In contrast to the development of visceral abscesses that may complicate septicemia due to typical *Y. enterocolitica* (33), localized skin abscesses (patient 15), rarely encountered with typical *Y. enterocolitica* (28), seem to be another manifestation of infection with biochemically and serologically atypical *Y. enterocolitica*. In this regard, Lewis and Alexander (26) documented a facial abscess due to a sucrose-negative *Y. enterocolitica*; Wilson and co-workers (39)

reported a labial abscess in a 4-month-old infant due to a serotype 0:20 *Y. enterocolitica*; and Lawrence and co-workers (24) described a furuncle on the inner aspect of the left thigh caused by a serotype 0:21 *Y. enterocolitica*. Unfortunately, since neither microbiological nor serological data accompanied the report of a patient with a lung abscess and osteomyelitis of a rib due to *Y. enterocolitica* (34), the exact nature of the infecting organism is unknown.

Of striking interest in the present study was the recovery of an atypical *Yersinia* from eight cases of conjunctivitis. The isolate was considered significant in six cases (patients 3, 4, 9, 14, 16, 19) and of questionable or of no significance in patients 6 and 17. A rhamnose-fermenting strain was isolated from the conjunctiva in pure culture in two instances (patient 4 and 9), with *S. epidermidis* on two other occasions (patient 3 and 16), and with a *Pseudomonas* species (patient 14). In each instance, conjunctivitis was present. The 14-month-old infant in case 19, from whom a sucrose-negative strain was isolated along with *E. cloacae*, developed bilateral conjunctivitis following 2 days of diarrhea. Although stool specimens were not submitted for culture, it is probable that the child's source of *Yersinia* was her own gastrointestinal tract. As noted herein and by Bissett (3), sucrose-negative isolates can be recovered from patients with acute enteritis.

Epidemiologically, the reservoir for these unusual *Yersiniae* remains unknown. The possibility exists that rhamnose-fermenting strains could have been introduced into the hospital and hence nosocomially acquired. Such a route of infection is highly suggested in the valuable report of Dabermat and associates (H. J. Dabermat, R. Bauriaud, J. Lemozy, J. C. Lefevre, and M. B. Lareng, 3rd International Symposium on Yersiniosis, Mont Gabriel, Canada-Saranac Lake, N.Y., 1977). These authors isolated 14 rhamnose-positive *Y. enterocolitica* from blood cultures of infants and children during a 7-month period. These patients were all hospitalized for an unrelated illness (meningococemia, listeriosis, group B streptococcal infection, etc.) for which they received parenteral medications. Extensive epidemiological studies conducted a minimum of 4 days after recovery of a strain failed to reveal a common source.

In our series, however, several factors seem to preclude nosocomial acquisition of the infecting strain. Although some clustering of isolates did occur, isolations spanned 4 years and were derived from patients who were seen mainly in outpatient clinics geographically dispersed throughout the institution. In one instance (patient 20), the patient may have acquired her

infection at the Day Care Center where she worked. Two staff members at this institution both had had diarrhea prior to the patient's first episode, and outbreaks of yersiniosis in collective care establishments for children have been described (32).

While an intrahospital focus seems uncertain, the possibility of a reservoir in the community served by The Mount Sinai Hospital does exist. Thirteen of these isolates were recovered from patients residing in the immediate vicinity of the hospital and one within a 1-mile radius. A cluster of four cases was derived from the lower East Bronx, while the remaining two cases, also from the Bronx, were spatially separated from each other by approximately 1 mile. Each area is composed of families from a lower socio-economic bracket, and overcrowding and poor sanitary conditions may prevail which could facilitate environment-to-human as well as interhuman transmission (17, 22).

According to Mollaret (30), it appears that there are two basic groups of *Y. enterocolitica*. The first is comprised of strains that have become well adapted in animal and human hosts and are biologically consistent with regard to cultural, biochemical, serological, and phage typing patterns. Examples include serotype 0:3, biotype 4, phage type 8, encountered predominantly in Europe and Japan; serotype 0:9, biotype 2, phage group 10 (nontypable), recovered almost exclusively in Scandinavian countries; and, in the United States, serotype 0:8, biotype 1, phage group 10. These strains are responsible for the "classical" syndromes associated with *Y. enterocolitica* (30).

The second group of *Y. enterocolitica*, as exemplified by the rhamnase-fermenting and, to a lesser extent, the sucrose-negative strains, is composed of members encountered mainly from environmental sources. These strains are characterized by their aberrant temperature-related cultural and biochemical profile, heterogeneous serotypes or inagglutinability with existing *Y. enterocolitica* antisera, lack of phage susceptibility, and wide geographic distribution. As indicated, rhamnase-fermenting varieties apparently are not as invasive as typical *Y. enterocolitica* and hence do not produce the more serious manifestations of yersiniosis. It may be inferred by the antibody response noted with patient 20 that sucrose-negative strains are intermediate in virulence between typical *Y. enterocolitica* and rhamnase-fermenting strains. According to Brenner and co-workers (10), rhamnase-fermenting and sucrose-negative strains form deoxyribonucleic acid homology groups distinct from typical *Y. enterocolitica*.

The role of biochemically atypical *Y. enter-*

ocolitica in human infections is slowly being elucidated. It is plausible that these exogenous strains have already become host adapted in humans but have been overlooked as a cause of pathology because of their distinctiveness from typical *Y. enterocolitica* and their overall resemblance to other *Enterobacteriaceae*. Alternatively, it is also plausible that these strains are presently in the evolutionary process of becoming host adapted in humans. Because of their genetic relatedness to *Enterobacteriaceae* (10), a potential exists for their acquisition of plasmids from other members' coding for colonization factors and/or enterotoxin production (15) which could markedly enhance their success in this adaptation. Only continued reports of their association with human disease will clarify their evolutionary pursuit.

ADDENDUM IN PROOF

Since the submission of this manuscript, a heat-labile enterotoxin has been demonstrated in 13% of rhamnase-fermenting *Y. enterocolitica* strains tested (C. H. Pai, V. Mors, and S. Toma, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C10, p. 278).

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