Temperature-Dependent Cultural and Biochemical Characteristics of Rhamnose-Positive *Yersinia enterocolitica*

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Clinical isolates of rhamnose-positive *Yersinia enterocolitica* (Y.e.^{rh+}) were compared with typical rhamnose-negative *Y. enterocolitica* (Y.e.^{rh-}) and with *Yersinia pseudotuberculosis*. The Y.e.^{rh+} differed from the Y.e.^{rh-} and *Y. pseudotuberculosis* in their ability to ferment raffinose and lactose, utilize citrate and in their inability to grow on Hektoen enteric agar at 22 or 37 C, on Salmonella-Shigella agar at 37 C, and scant growth on xylose-lysine-deoxycholate agar at 37 C. An extensive temperature-dependent profile of characteristics was established for the Y.e.^{rh+}: motility, acetoin production, citrate utilization, growth on Salmonella-Shigella agar, and ampicillin resistance occurred at 22 C but not 37 C; fermentation of melibiose, raffinose, and cellobiose occurred within 24 h at 22 C, but not before 5 days at 37 C; fermentation of rhamnose and production of beta-galactosidase occurred within 24 h at 22 C, but not before 48 h at 37 C; greater resistance to ampicillin, chloramphenicol, streptomycin, kanamycin, carbenicillin, and gentamicin was observed at 22 than 37 C; and good growth on xylose-lysine-deoxycholate agar occurred at 22 but not 37 C. For optimal recovery of Y.e.^{rh+} from mixed culture, e.g., stools, two MacConkey plates should be inoculated and incubated, one at 37 C, and one at 22 C. Lactose-negative colonies appearing after 48 h on the 22 C MacConkey agar but not the 37 C MacConkey agar should be considered possible Y.e.^{rh+}. Biochemicals should be tested in duplicate, one set incubated at 22 C, one set at 37 C. Antibiotic susceptibility tests of Y.e.^{rh+} isolates should be incubated at both 37 C and at a lower temperature to allow the greatest expression of resistance of these organisms to the various antibiotics.

During a 2-year period (1971-1973), 12 rhamnose-fermenting organisms, identified as *Yersinia enterocolitica*, were recovered from a variety of clinical specimens submitted to the clinical microbiology laboratory at the Mount Sinai Hospital, New York. A preliminary investigation of the biochemical characteristics and growth capabilities of these isolates revealed marked differences from *Y. enterocolitica* serotypes 1 through 16 (4). Although at least three antigenic groups were present among the 12 isolates, all appeared to be identical, biochemically and culturally, with *Y. enterocolitica*, serotype 17, isolated by Lassen in Denmark from fecally contaminated drinking water (8). Although no additional reports of rhamnose-positive *Y. enterocolitica* (Y.e.^{rh+}) strains from human sources have appeared, similar organisms have been isolated from water sources in the United States and from Canadian Lakes (11).

This report represents an investigation of the biochemical and cultural characteristics of

Y.e.^{rh+} isolates and compares them with those of rhamnose-negative *Y. enterocolitica* (Y.e.^{rh-}) and *Yersinia pseudotuberculosis* for the purpose of differentiating these three organisms from each other. Special attention was paid to the temperature-dependent features, in addition to motility, observed with Y.e.^{rh+} but not with Y.e.^{rh-} or *Y. pseudotuberculosis* isolates. This information was used in the isolation, identification, and antibiotic susceptibility testing of Y.e.^{rh+} isolates.

MATERIALS AND METHODS

The 12 Mount Sinai isolates of Y.e.^{rh+}, referred to as strains 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 59, and 60, were recovered from the following specimens: eye (4), stool (3), urine (2), throat (1), trachea (1), and wound (1). An additional Y.e.^{rh+} belonging to serotype 17 (strain 17) and 16 Y.e.^{rh-} isolates representing serotypes 1 through 16, were kindly provided by S. Winblad, Malmö, Sweden. The 10 *Y. pseudotuberculosis* strains examined were supplied by Analylab Products, Inc. (Plainview, Long Island).

Motility was determined by dark-field microscopy (×970) examination of hanging-drop preparations of 18-h Tryptic-soy broth (TSB; Scott Laboratories,

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Providence, R.I.) cultures incubated at 22 and 37 C, respectively.

Growth capabilities on enteric agars were determined using MacConkey (MC), xylose-lysine-deoxycholate (XLD), Salmonella-Shigella (SS, all BBL), and Hektoen enteric (HE; Difco) agars. The test organisms were grown in TSB at 37 C until the turbidity equaled an optical density (OD) of 0.350 at 420 nm. One loopful of broth culture was streaked to eight plates (two each of XLD, HE, MC, and SS agars). Streaking was accomplished by moving the loop across one-third of the agar surface (area I) flaming, drawing out some of the inoculum of area I and streaking it across a second third of the agar surface (area II), flaming, and repeating in the third area (III). One plate of each agar type was incubated at 22 C and the second plate of each agar type was incubated at 37 C. For each organism, this procedure was performed three times. All plates were examined after 24 and 48 h of incubation. Growth was considered positive by the presence of colonies, in areas II and III, visible to the unaided eye.

Growth curves were constructed to explore the possibility of a growth advantage of *Y.e.*^{rh+} at 22 C as an explanation of the many temperature-dependent characteristics of these organisms, as described in this report. These curves were obtained at 22 and 37 C for *Y.e.*^{rh+} strain 17, *Y.e.*^{rh-} serotype 8, and *Y.pseudotuberculosis*. Two 18-h TSB cultures of each organism were used as the starting inocula, one having been incubated at 37 C and the other at 22 C. Flasks inoculated with broth culture incubated at 37 C were, in turn, incubated at 37 C; flasks inoculated with broth cultures incubated at 22 C were incubated at 22 C. Before incubation, samples were removed and examined for viable cell counts using Standard Methods agar (Difco) pour plates and OD₄₂₀ readings. Viable cell counts and OD₄₂₀ readings were then made after 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 h.

Mixed culture studies were performed to determine the incubation conditions necessary for optimal recovery of the *Y.e.*^{rh+}, especially from stools. Using 18-h TSB cultures, 10 loopfuls of *Escherichia coli*, one loopful of *Proteus morganii*, and one loopful of *Y.e.*^{rh+} strain 17 were inoculated into 5 ml of sterile saline and then mixed. Each of the 12 remaining *Y.e.*^{rh+} isolates was similarly mixed with *E. coli* and *P. morganii*. Each of these 13 mixtures was then plated on two MC plates, one incubated at 22 C and the other at 37 C, and then examined after 24 h for isolated lactose-negative colonies. Two of these colonies, from each plate, were transferred to Christensen urea agar and to 1% arabinose broth (Scott). The MC plates were incubated for an additional 24 h, after which the transfer procedure was repeated. A colony producing a positive urease reaction and fermenting arabinose was considered to be a presumptive *Y. enterocolitica* isolation. To confirm the isolation as *Y. enterocolitica*, rather than a possible mixture of *E. coli* and *P. morganii*, which would also produce positive arabinose and urea tests, the positive arabinose broth was streaked to a fresh MC plate and incubated at 37 C for 24 h to detect the presence of any lactose-positive *E. coli* colonies. This

procedure was performed five times with each of the 13 freshly prepared *E. coli*-*Y. enterocolitica*-*P. morganii* mixtures.

Biochemical tests were performed at 22 and 37 C as outlined by Edwards and Ewing (5), unless otherwise noted. All substrates were inoculated with test organisms grown on 5% sheep blood agar at 37 C for 18 h. All tests were examined daily. Negative tests were held for 14 days before being discarded (exceptions are noted). Broth base containing phenol red indicator and a 1% (wt/vol) concentration of carbohydrate were used for fermentation studies involving glucose, lactose, sucrose, maltose, raffinose, rhamnose, cellobiose, melibiose, salicin, sorbitol, arabinose, mannitol, dulcitol, xylose, and trehalose (Scott). A change of the phenol red indicator to a distinct lemon-yellow color was interpreted as fermentation of the test carbohydrate. Esculin fermentation was determined using enterococcosel agar (BBL) (9). Decarboxylation of lysine and ornithine and dihydrolyation of arginine were determined with Moellers formulation modified to a semisolid agar (0.4%) (Scott). Deamination of phenylalanine was detected with phenylalanine agar slants (Scott). All amino acid degradation tests considered negative were discarded after 4 days. Urease activity was studied on Christensen urea agar. Indol production was tested with SIM medium (Scott), using Kovac reagent as the indicator. Acetoin production from acetyl-methylcarbinol (Voges-Proskauer) was tested with buffered peptone-glucose broth (Scott) after a minimum of 24 h of incubation using the Barritt modification (9). Tests for beta-galactosidase activity were performed by using tablets containing *O*-nitrophenol-beta-D-galactopyranoside (Key Scientific Products Co., Los Angeles). For oxidase tests, oxidase strips (PathoTec C-O test strips, Warner Lambert, Morris Plains, N.J.) were used.

Antibiotic susceptibility tests were performed by the disk-diffusion method of Bauer et al. (3). The following antibiotic disks were tested: ampicillin (10 µg), carbenicillin (50 µg), cephalothin (30 µg), chloramphenicol (30 µg), colistin (10 µg), gentamicin (10 µg), kanamycin (30 µg), polymyxin (300 U), streptomycin (10 µg), and tetracycline (30 µg) (BBL). Each organism was tested at 22 and 37 C. Zones of inhibition were interpreted as susceptible or resistant based on interpretative tables recommended by the Food and Drug Administration (6, 7).

Serological typing of the *Y.e.*^{rh+} strains was accomplished by tube agglutination titrations of rabbit antiserum with antigen preparation from each *Y.e.*^{rh+} strain according to the method of Winblad et al. (12). *Y.e.*^{rh+} strain 57 was kindly confirmed as *Y. enterocolitica* serotype 16 by H. Mollaret of L'Institut Pasteur, Paris.

RESULTS

The growth curves of *Y.e.*^{rh+} strain 17 and *Y.e.*^{rh-} serotype 8 were essentially the same. At 22 C the curves of these two organisms paralleled their 37 C curves, except that an additional hour was required to reach the logarithmic phase (Fig. 1 and 2). Although the *Y. pseu-*

FIG. 1. Growth of a culture of *Yersinia enterocolitica* (*Y.e.*^{rh+}) strain 17 in TSB at 37 C (solid line) and at 22 C (dotted line) during a 24-h period.

FIG. 2. Growth of a culture of *Y. enterocolitica* (*Y.e.*^{rh-}) serotype 8 in TSB at 37 C (solid line) and at 22 C (dotted line) during a 24-h period.

dotuberculosis isolate demonstrated a slower growth rate than did the *Y.e.*^{rh+} and *Y.e.*^{rh-} isolates, no significant difference in growth rate was observed when grown at 22 and 37 C (Fig. 3). After various incubation periods, the colony counts (Table 1) and the growth curves demonstrated that, other than a 1-h lag in reaching logarithmic growth, no significant variation occurred in the rate of growth of these three *Yersinia* organisms at 22 and 37 C. Certainly, no growth advantage at 22 C was observed with the *Y.e.*^{rh+} strain 17, invalidating such an advantage as an explanation for the many temperature-dependent characteristics of this organism. On the contrary, colony counts for *Y.e.*^{rh+} isolate 17 were lower at 22 C than for each time interval sampled (Table 1).

Of the four enteric agars tested, only MC and XLD supported the growth of all *Y.e.*^{rh+}, *Y.e.*^{rh-}, and *Y. pseudotuberculosis* isolates. With the exception of three *Y.e.*^{rh+} isolates, which failed to grow at 22 or 37 C after 48 h, all isolates grew on SS agar (Table 2). HE agar was least effective in supporting growth of the three types of *Yersinia*: 12 of 13 *Y.e.*^{rh+} isolates did not grow at 22 or 37 C, nor did one of the 10 *Y. pseudotuberculosis* isolates (Table 3). Of the three types of *Yersinia* studied, the *Y.e.*^{rh-} grew most readily and on the greatest variety of enteric agars (Table 4).

The growth of *Y.e.*^{rh+} on XLD and SS showed a temperature dependence. Whereas all 13 isolates grew on XLD incubated at 22 C for 48 h, only five grew at 37 C and, in each instance, the colonies were less than 0.5 mm in diameter (i.e., pinpoint). On SS, nine of the *Y.e.*^{rh+} iso-

lates grew as pinpoint colonies when incubated at 22 C for 48 h but only one did so at 37 C (Table 2).

The failure of any of the *Y.e.*^{rh+} isolates to produce colonies greater than 0.5 mm in diameter or, in most cases, to grow at all on SS and HE at 37 or 22 C or on XLD at 37 C, was in sharp contrast to the *Y.e.*^{rh-} and *Y. pseudotuberculosis* isolates, most of which produced colonies of a diameter greater than 0.5 mm, at 37 and 22 C, on these three agars. One exception, as previously noted, was the poor growth of one isolate of *Y. pseudotuberculosis* on HE. The inability or limited ability of *Y.e.*^{rh+} to grow on three of the most commonly used enteric agars demonstrated that the isolation of this organism from stool cultures, using enteric agars, such as XLD, HE, and SS, but not MC, and incubation at 37 C, was improbable, or more likely, impossible.

The isolation of the *Y.e.*^{rh+} isolates from at least four of the five attempts with each of the 13 experimental mixtures was considered a successful recovery (Table 5). Using this criterion, the highest recovery rates (essentially 100%) were obtained when the mixtures were plated on MC and isolated lactose-negative colonies chosen after incubation at 22 C for 48 h. *Y.e.*^{rh+} isolates, however, were not isolated from at least four of five attempts from any of the 13 different mixtures when incubation at 22 C for only 24 h or when incubated at 37 C for 48 h. Inasmuch as most clinical microbiology laboratories incubate enteric agar plates (e.g., MC) at 37 C rather than at 22 C, most infections caused by *Y.e.*^{rh+} and some by *Y.e.*^{rh-} have probably escaped detection, as evidenced by the scarcity of reported cases.

All isolates of *Y.e.*^{rh+}, *Y.e.*^{rh-}, and *Y. pseudotuberculosis* were motile at 22 C but not at 37 C when examined by the hanging-drop technique.

The *Y.e.*^{rh+} isolates possessed the basic biochemical characteristics of the other members of the family *Enterobacteriaceae* which are fermentation of glucose, reduction of nitrate to

FIG. 3. Growth of a culture of *Y. pseudotuberculosis* isolate 286 in TSB at 37 C (solid line) and at 22 C (dotted line) during a 24-h period.

TABLE 1. Colony counts per milliliter of *Y. enterocolitica* (*Y.e.*^{rh+}) strain 17, *Y. enterocolitica* isolate 8 and *Y. pseudotuberculosis* isolate 286 in TSB at 37 and 22 C during a 24-h period

Time of incubation (h)	<i>Y.e.</i> ^{rh+} strain 17		<i>Y. enterocolitica</i> serotype 8		<i>Y. pseudotuberculosis</i>	
	37 C	22 C	37 C	22 C	37 C	22 C
0	8.9×10^6	8.7×10^6	8.7×10^6	8.6×10^6	8.6×10^6	8.2×10^6
1	1.0×10^7	1.1×10^7	9.5×10^6	8.6×10^6	8.3×10^6	8.9×10^6
2	1.9×10^7	1.0×10^7	1.7×10^7	9.5×10^6	8.7×10^6	8.8×10^6
3	3.6×10^7	1.4×10^7	2.7×10^7	1.5×10^7	1.5×10^7	9.1×10^6
4	1.2×10^8	4.7×10^7	8.4×10^7	4.4×10^7	2.9×10^7	2.0×10^7
5	2.3×10^8	1.4×10^8	2.1×10^8	9.7×10^7	7.3×10^7	3.4×10^7
6	5.7×10^8	2.3×10^8	5.2×10^8	2.1×10^8	2.0×10^8	8.0×10^7
7	8.6×10^8	3.1×10^8	8.7×10^8	3.7×10^8	2.9×10^8	1.3×10^8
8	1.5×10^9	1.3×10^9	1.2×10^9	8.0×10^8	6.0×10^8	2.9×10^8
12	9.9×10^8	7.0×10^8	1.0×10^9	1.2×10^9	9.7×10^8	8.8×10^8
24	9.7×10^8	9.5×10^8	9.2×10^8	8.9×10^8	8.8×10^8	8.6×10^8

TABLE 2. The ability of 13 *Y. enterocolitica* (*Y.e.*^{rh+}) isolates to grow on commonly used enteric agars under different incubation conditions^a

<i>Y.e.</i> ^{rh+} strains	MC agar				XLD				SS agar				HE agar			
	22 C		37 C		22 C		37 C		22 C		37 C		22 C		37 C	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
17	+		+		-	(+)	-	-	-	(+)	-	-	-	-	-	-
48	+		+		-	+	-	-	-	-	-	-	-	-	-	-
49	+		+		-	+	-	(+)	-	(+)	-	(+)	-	(+)	-	(+)
50	+		+		-	+	-	-	-	(+)	-	-	-	-	-	-
51	+		+		-	+	-	-	-	(+)	-	-	-	-	-	-
53	+		+		-	+	-	-	-	(+)	-	-	-	-	-	-
54	+		+		-	+	-	-	-	(+)	-	(+)	-	-	-	-
55	+		+		-	+	-	(+)	-	-	-	-	-	-	-	-
56	+		+		-	+	-	(+)	-	-	-	-	-	-	-	-
57	+		+		-	+	-	(+)	-	(+)	-	-	-	-	-	-
58	+		+		-	+	-	-	-	(+)	-	-	-	-	-	-
59	+		+		-	+	-	-	-	-	-	(+)	-	-	-	-
60	+		+		-	+	-	(+)	-	(+)	-	-	-	-	-	-

^a +, Colonies of at least 0.5 mm diameter; -, no visible growth; (+), colonies of less than 0.5 mm diameter (i.e., pinpoint).TABLE 3. The ability of 10 *Y. pseudotuberculosis* isolates to grow on commonly used enteric agars under different incubation conditions^a

<i>Y. pseudotuberculosis</i> isolate	MC agar				XLD				SS agar				HE agar			
	22 C		37 C		22 C		37 C		22 C		37 C		22 C		37 C	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
286	-	+	+		-	+	-	+	-	+	-	(+)	+		(+)	+
287	+		+		-	+	-	+	-	+	-	+	-	(+)	-	-
288	-	+	+		-	+	-	+	-	(+)	-	(+)	(+)	+	-	(+)
289	-		-	+	-	+	-	(+)	-	(+)	-	+	-	(+)	-	-
290	-		-	+	-	(+)	-	+	-	-	-	+	-	(+)	-	(+)
291	-		-	+	-	+	-	+	-	-	-	+	-	-	-	-
293	-		-	+	-	+	-	+	-	(+)	-	+	(+)	+	-	-
294	-		-	+	-	+	-	+	-	(+)	-	-	(+)	+	-	(+)
295	-		-	+	-	+	-	+	-	(+)	-	-	(+)	+	-	-
296	-		-	+	-	+	-	+	-	+	-	(+)	-	(+)	-	-

^a -, No visible growth; +, colonies of at least 0.5 mm diameter; (+), colonies of less than 0.5 mm diameter (i.e., pinpoint).

nitrite, production of catalase, and an absence of oxidase activity. In addition to being motile at 22 C but not at 37 C, each of the *Y.e.*^{rh+} isolates exhibited the profile of biochemical

characteristics common to *Y. enterocolitica* and *Y. pseudotuberculosis* and which distinguished these two species from the other *Enterobacteriaceae*: production of urease and beta-galac-

TABLE 4. Ability of 16 *Y. enterocolitica* (*Y.e.*^{rh-}) serotypes to grow on commonly used enteric agars under different incubation conditions^a

<i>Y.e.</i> ^{rh-}	MC agar				XLD				SS agar				HE agar			
	22 C		37 C		22 C		37 C		22 C		37 C		22 C		37 C	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1	+		+		+		+		-	+	+		+		+	
2	+		+		-	+	+		-	+	+		(+)	(+)	(+)	(+)
3	+		+		+		+		+		+		+		+	
4	+		+		+		+		+		+		+		+	
5	+		+		+		+		-	(+)	-	(+)	+		+	
6	+		+		-	(+)	-	(+)	-	-	-	(+)	-	(+)	-	(+)
7	+		+		+		+		-	+	-	(+)	+		+	
8	+		+		-	+	+		+		+		+		+	
9	+		+		+		+		+		+		+		+	
10	+		+		-	+	+		+		+		+		+	
11	+		+		+		+		+		+		(+)	(+)	(+)	(+)
12	+		+		-	+	+		+		-	(+)	-	(+)	-	(+)
13	+		+		+		+		+		-	(+)	+		+	
14	+		+		+		+		+		-	(+)	+		+	
15	+		+		+		+		+		+		+		+	
16	+		+		+		+		+		-	(+)	+		+	

^a +, Colonies of at least 0.5 mm diameter; -, no viable growth; (+), colonies of less than 0.5 mm diameter (i.e., pinpoint).

TABLE 5. Rate of recovery of *Y. enterocolitica* (*Y.e.*^{rh+}) strains from mixed culture on MC agar under different incubation conditions^a

<i>Y.e.</i> ^{rh+} strain	22 C		37 C	
	24 h	48 h	24 h	48 h
17	0 ^b	4	0	0
48	0	4	0	3
49	1	5	0	3
50	0	5	0	0
51	3	5	3	2
53	1	5	0	1
54	0	5	1	2
55	1	4	0	0
56	3	5	0	1
57	3	5	3	3
58	0	5	0	3
59	0	4	0	0
60	1	4	0	0
Recovery rate ^c	0	100	0	0

^a Mixed cultures contained *Y.e.*^{rh+}, *E. coli*, and *P. morganii*: 1:10:1.

^b Numerical value, number of *Y.e.*^{rh+} isolates recovered during five attempts.

^c A recovery was considered to be positive when a *Y.e.*^{rh+} strain was recovered in at least four of five attempts.

tosidase; fermentation of maltose, mannitol, arabinose, xylose, trehalose, esculin, and salicin; failure to ferment dulcitol; lack of activity of lysine decarboxylase, phenylalanine deaminase, and arginine dihydrolase; and inability to produce hydrogen sulfide detectable in Kligler iron agar or in SIM medium.

Like *Y.e.*^{rh-}, but unlike *Y. pseudotubercu-*

losis, the *Y.e.*^{rh+} isolates decarboxylated ornithine, fermented sucrose, sorbitol, and cellobiose, failed to ferment adonitol, and produced a positive Voges-Proskauer reaction (acetoin production) at 22 C but not at 37 C. All *Y.e.*^{rh+} isolates fermented rhamnose and melibiose, two features shared by *Y. pseudotuberculosis* but not by *Y.e.*^{rh-}; however, the *Y.e.*^{rh+} isolates differed from both *Y.e.*^{rh-} and *Y. pseudotuberculosis* in their ability to utilize citrate as a sole carbon source and to ferment raffinose. Additionally, the *Y.e.*^{rh+} isolates were lactose-positive, in contrast to all *Y.e.*^{rh-} and *Y. pseudotuberculosis* isolates studied. However, a minimum of 8 days was required by the *Y.e.*^{rh+} isolates to ferment lactose at either 22 or 37 C. Indol was produced by all of the *Y.e.*^{rh+} isolates, whereas the *Y.e.*^{rh-} isolates were variable, and all *Y. pseudotuberculosis* isolates were indol negative (Table 6).

Although each of the isolates of the three types of *Yersinia* studied was motile at 22 C but not at 37 C, and all *Y.e.*^{rh+} and *Y.e.*^{rh-} isolates produced positive Voges-Proskauer tests at 22 C but not at 37 C, only the *Y.e.*^{rh+} isolates were found to have the following additional temperature-dependent characteristics: citrate utilization, fermentation of melibiose, raffinose, cellobiose, and rhamnose, and beta-galactosidase activity were greater at 22 than at 37 C (Table 7). When incubated at 37 C for 14 days, none of the *Y.e.*^{rh+} isolates grew on Simmon citrate agar but, at 22 C, eight grew within 24 h, and the remaining five grew within 48 h. Cellobiose was fermented within 24 h at 22 C by each of the *Y.e.*^{rh+} isolates, whereas at 37 C,

TABLE 6. Biochemical differentiation of *Y. enterocolitica* (*Y.e.*^{rh+}), *Y. enterocolitica* (*Y.e.*^{rh+}), and *Y. pseudotuberculosis*

Test or substrate	<i>Y.e.</i> ^{rh+}	<i>Y.e.</i> ^{rh-}	<i>Y. pseudotuberculosis</i>
Citrate			
22 C	+	-	-
37 C	-	-	-
Fermentation of raffinose			
22 C	+	-	-
37 C	(+)	-	-
Lactose			
22 C	(+)	-	-
37 C	(+)	-	-
Melibiose			
22 C	+	-	+
37 C	(+)	-	+
Rhamnose			
22 C	+	-	+
37 C	+	-	+
Cellobiose			
22 C	+	+	-
37 C	d	+	-
Adonitol			
22 C	-	-	+
37 C	-	-	+
Sucrose			
22 C	+	+	-
37 C	+	+	-
Sorbitol			
22 C	+	+	-
37 C	+	+	-
Ornithine			
22 C	+	+	-
Voges-Proskauer			
22 C	+	+	-
37 C	-	-	-
Indol (SIM)			
37 C	+	d ^b	-
Pink reaction			
37 C	+	d ^c	-

^a +, 90% or more positive within 2 days; -, 90% or more negative within 2 days; (+), 90% or more positive within 14 days but not before 3 days; d, different reactions: +, (+), -.

^b *Y.e.*^{rh-} serotypes 1, 2, 3, 11, and 12 were negative.

^c *Y.e.*^{rh-} serotypes 1, 2, 3, and 15 were negative.

although six were also positive after 24 h, four required from 5 to 8 days and three of the isolates remained cellobiose negative after 14 days of incubation. Melibiose was fermented within 24 h at 22 C by all *Y.e.*^{rh+} isolates but, at 37 C, 5 to 8 days was needed. All *Y.e.*^{rh+} fermented rhamnose in 24 h at 22 C, but eight isolates required 48 h at 37 C. Beta-galactosidase activity was detected with all 13 isolates within 8 h at 22 C but, at 37 C activity was not detected for 24 h with seven of the isolates and 48 h with the remaining six.

The ability of the *Y.e.*^{rh+} isolates to utilize citrate at 22 C and to ferment raffinose, rhamnose, and lactose at 37 and 22 C formed the basis of a biochemical profile for these organisms which placed them apart from the *Y.e.*^{rh-} and *Y. pseudotuberculosis* isolates studied. These differences can provide the clinical microbiology laboratory with a simple, reliable method for properly identifying an isolate as a *Y.e.*^{rh+}.

The *Y. pseudotuberculosis* isolates were the most sensitive of the three *Yersinia* types; all 10 isolates were sensitive to each of the antibiotics tested, with the exception of two which were resistant to colistin.

The *Y.e.*^{rh-} isolates were the most resistant of the three *Yersinia* types. Although uniformly sensitive to streptomycin, tetracycline, chloramphenicol, kanamycin, polymyxin B, colistin, and gentamicin, resistance to ampicillin, cephalothin, and carbenicillin was noted with all *Y.e.*^{rh-} isolates, except 8 and 11. *Y. enterocolitica* isolate 12, although sensitive to carbenicillin, was also resistant to ampicillin and cephalothin.

The *Y.e.*^{rh+} isolates demonstrated a temperature-dependent relationship in their ability to resist the bacteriostatic effect of chloramphenicol and the bactericidal effect of streptomycin, ampicillin, kanamycin, carbenicillin, and gentamicin (Table 8). Inhibition zones at 37 C were often 5 mm or greater than at 22 C when involving the same isolate and the same antibiotic, disregarding variations to 4 mm in diameter in allowance for such factors as visual acuity, lighting, and variations in batches of media. This was especially apparent with streptomycin, against which nine of the 13 *Y.e.*^{rh+} isolates had inhibition zones that were greater by 5 mm at 37 C than at 22 C, and with gentamicin, against which 10 of the *Y.e.*^{rh+} isolates had inhibition zones that were greater by 5 mm or more at 37 C than at 22 C.

An important consequence of the temperature-dependent antibiotic susceptibility relationship of the *Y.e.*^{rh+} isolates was noted with ampicillin. Although each of the 13 *Y.e.*^{rh+} isolates was sensitive to ampicillin at 37 C, six of the isolates, 48, 49, 50, 51, 53, and 55, had inhibition zones at 22 C which fell into the resistant category. This suggested that antibiotic susceptibility tests of *Y.e.*^{rh+} isolates should be incubated at 22 C to allow the greatest expression of resistance of the organism to antibiotics. A similar proposal has been made for the testing of methicillin-resistant *Staphylococcus aureus* isolates (9).

Serological typing of the 13 *Y.e.*^{rh+} strains demonstrated three groups. The first consisted

TABLE 7. Temperature-dependent characteristics of 13 *Y. enterocolitica* (Y.e.^{rh+}) strains

Y.e. rh+ strain	Motility		Citrate		Voges-Proskauer		Carbohydrate fermentation								Beta-galactosidase	
							Melibiose		Raffinose		Cellobiose		Rhamnose			
	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C
17	+	—	1	—	2	—	1	5	1	5	1	1	1	1	<1	2
48	+	—	1	—	2	—	1	6	1	7	2	8	1	2	<1	2
49	+	—	1	—	2	—	1	5	1	4	2	5	1	2	<1	2
50	+	—	1	—	2	—	1	6	1	9	2	8	1	2	<1	1
51	+	—	1	—	2	—	1	6	1	5	2	—	1	2	<1	1
53	+	—	2	—	2	—	1	8	1	8	2	8	1	2	<1	2
54	+	—	2	—	2	—	1	6	1	7	2	—	1	2	<1	1
55	+	—	1	—	2	—	1	6	1	6	2	—	1	2	<1	1
56	+	—	2	—	2	—	1	5	1	4	1	1	1	1	<1	1
57	+	—	1	—	2	—	1	8	1	4	1	1	1	2	<1	1
58	+	—	1	—	2	—	1	5	1	4	1	1	1	1	<1	2
59	+	—	2	—	2	—	1	5	1	4	1	1	1	1	<1	2
60	+	—	2	—	2	—	1	5	1	5	1	1	1	1	<1	1

+ , Positive for test; —, negative for test; numerical value, number of days required for positive test.

of strains 17, 48, 49, 50, 51, 53, 54, and 59, all of which were members of *Y. enterocolitica* serotype 17. The second group was represented by strain 57 and conformed to *Y. enterocolitica* serotype 16. The third group contained 55, 56, 58, and 60, none of which conformed to any of the known serological groups of *Y. enterocolitica*.

DISCUSSION

Although the 13 bacterial isolates studied and referred to as Y.e.^{rh+} possessed the basic biochemical profile of Y.e.^{rh-} and *Y. pseudotuberculosis*, the Y.e.^{rh+} isolates differed significantly in other characteristics from the 16 Y.e.^{rh-} and the 10 *Y. pseudotuberculosis* isolates studied. Only the Y.e.^{rh+} were capable of fermenting raffinose and lactose and of utilizing citrate as a sole source of carbon. Although lactose-positive, the 8-day delay in fermentation by Y.e.^{rh+} isolates prevented their colonies from appearing as lactose-positive on MC during the initial 48-h examination period. The ability of rhamnose-positive *Y. enterocolitica* to ferment rhamnose and melibiose 1 to 7 days earlier and to ferment cellobiose as much as 6 days earlier at 22 C incubation than at 37 C was further evidence of its distinctiveness from Y.e.^{rh-} (negative rhamnose and melibiose) and *Y. pseudotuberculosis* (cellobiose negative) which fermented rhamnose and melibiose with equal facility at both incubation temperatures (Table 7).

In addition to stressing further the distinctiveness of the Y.e.^{rh+} isolates from Y.e.^{rh-} and *Y. pseudotuberculosis*, a knowledge of temperature-dependent biochemical features is required to properly identify a clinical isolate as a Y.e.^{rh+}. Inasmuch as most, if not all, clinical laboratories incubate biochemical tests at 37 C

and discard these tests within 24 to 48 h, many of the biochemical characteristics salient to the proper identification of Y.e.^{rh+} will be scored incorrectly, resulting in misidentification. It is suggested that all isolates suspected of being a species of *Yersinia* be tested in duplicate, one set of tests being incubated at 37 C and the second set at 22 C.

The temperature-dependent ability of the rhamnose-positive Y.e.^{rh+} isolates to grow on enteric agars can be considered one of their most important temperature-dependent features, as failure to isolate the organism obviates the need for identification and susceptibility testing. Incubation at 22 C was required for the cultivation of Y.e.^{rh+} from pure culture when using XLD and SS agars and for its recovery from mixed bacterial populations, such as found in stool cultures, even when using MC agar. At 37 C, the Y.e.^{rh+} strains grew poorly, or, in most instances, not at all on XLS, SS, or HE agar. Recovery of Y.e.^{rh+} using MC agar for examination of mixed cultures fell from 100% at 22 C to no recovery at all at 37 C. It is suggested, therefore, that stool cultures be tested in duplicate, with the inclusion of two MC plates in addition to the more commonly used agars, i.e., XLD, SS, and HE, and that one plate be incubated at 22 C and the other at 37 C. Any lactose-negative colony, present on the plate incubated at 22 C but not on the plate incubated at 37 C, should be suspected as a possible Y.e.^{rh+} and further studied. The data in Table 4 suggest that this procedure may aid in the recovery of Y.e.^{rh-} isolates as well.

Susceptibility of the Y.e.^{rh+} isolates to chloramphenicol, streptomycin, kanamycin, carbenicillin, gentamicin, and, especially, ampicillin was decreased when tested at 22 C. At this temperature, six of the 13 Y.e.^{rh+} isolates ex-

TABLE 8. Temperature-dependent, antibiotic susceptibility relationship of 13 *Y. enterocolitica* (*Y.e.*^{rt+}) strains

<i>Y.e.</i> ^{rt+} strain	St ^c (15) ^a		Te(19)		Am(14)		Cp(18)		C(18)		K(18)		Pb(12)		Cl(11)		Cb(23)		Gm(13)	
	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C	37 C	22 C
17	29-S	21-S	27-S	28-S	27-S	15-S	18-S	18-S	36-S	29-S	31-S	30-S	18-S	17-S	16-S	16-S	29-S	22-R	28-S	26-S
48	30-S	23-S	31-S	29-S	21-S	12-R	24-S	20-S	29-S	29-S	33-S	29-S	19-S	18-S	18-S	16-S	20-R	15-R	29-S	26-S
49	28-S	22-S	31-S	29-S	17-S	12-R	20-S	20-S	30-S	32-S	33-S	30-S	20-S	18-S	19-S	17-S	18-R	16-R	33-S	26-S
50	31-S	24-S	34-S	27-S	15-S	12-R	18-S	19-S	30-S	33-S	39-S	30-S	14-S	17-S	12-S	15-S	18-R	15-R	31-S	29-S
51	27-S	23-S	28-S	29-S	17-S	13-R	20-S	20-S	32-S	31-S	32-S	29-S	13-S	11-R	12-S	10-R	8-R	17-R	32-S	24-S
53	26-S	24-S	31-S	26-S	18-S	11-R	18-S	19-S	30-S	27-S	34-S	28-S	19-S	17-S	17-S	16-S	18-R	16-R	33-S	25-S
54	27-S	23-S	29-S	29-S	17-S	15-S	20-S	20-S	32-S	34-S	34-S	30-S	14-S	14-S	12-S	12-S	18-R	16-R	34-S	26-S
55	28-S	24-S	27-S	28-S	18-S	13-R	20-S	21-S	30-S	29-S	34-S	30-S	18-S	18-S	17-S	16-S	16-R	17-R	34-S	27-S
56	27-S	21-S	28-S	28-S	18-S	17-S	22-S	25-S	34-S	32-S	33-S	29-S	20-S	19-S	17-S	17-S	17-R	21-R	32-S	27-S
57	28-S	22-S	28-S	30-S	28-S	18-S	23-S	23-S	35-S	31-S	32-S	29-S	18-S	18-S	16-S	16-S	19-R	21-R	32-S	27-S
58	28-S	21-S	28-S	29-S	17-S	15-S	19-S	22-S	33-S	30-S	33-S	30-S	18-S	17-S	17-S	16-S	16-R	17-R	32-S	25-S
59	29-S	21-S	27-S	28-S	16-S	16-S	19-S	22-S	33-S	26-S	33-S	29-S	17-S	16-S	16-S	16-S	16-R	17-R	32-S	25-S
60	28-S	21-S	25-S	27-S	18-S	16-S	20-S	22-S	37-S	30-S	33-S	27-S	18-S	17-S	17-S	16-S	17-R	16-R	32-S	25-S

^a St, Streptomycin; Te, tetracycline; Am, ampicillin; Cp, cephalothin; C, chloramphenicol; K, kanamycin; Pb, polymyxin B; Cl, colistin; Cb, carbenicillin; Gm, gentamicin.

^b Numerical value in parentheses indicates minimum diameter (millimeters) of inhibition zone for an interpretation of sensitive, -S; a diameter smaller than the value indicated in parentheses is interpreted as resistant, -R.

hibited such decreased susceptibility to ampicillin as to be resistant, even though testing of the same isolates at 37 C indicated sensitivity to ampicillin. This temperature-dependent expression of resistance to antibiotics suggests that antibiotic susceptibility testing of *Y.e.*^{rh+} isolates, at least when results are ambiguous at 37 C, be performed at a lower temperature.

The results of studies of the growth of *Y.e.*^{rh+} isolates in broth eliminated the possibility that an increased growth rate at 22 C was responsible for the apparent temperature-dependent characteristics as there was slightly better growth at 37 C. Growth curves of *Y.e.*^{rh-} and *Y. pseudotuberculosis*, neither of which demonstrated the extensive temperature-dependent nature of the *Y.e.*^{rh+}, were essentially the same as those of the *Y.e.*^{rh+} isolates. It is possible that a temperature-dependent derepression occurs on those portions of the genome of *Y.e.*^{rh+} that code for the production of enzymes involved in the temperature-dependent characteristics, as for example, production of beta-lactamase (personal communication, E. J. Bottone, Mount Sinai Hospital, N.Y.).

Each of the 13 *Y.e.*^{rh+} isolates (with the exception of strain 17) was cultured from persons living in the vicinity of Mt. Sinai Hospital, New York. This area is economically depressed and exhibits the problems of such an area, namely rodent infestation, poor sanitary facilities and procedures, and inadequate diet. It is possible that a reservoir of *Y.e.*^{rh+} exists in the Mount Sinai area, perhaps in water sources (1, 2, 10, 13) and foods contaminated by this water, e.g., ice cream sold by street vendors using contaminated ice (2, 10).

We are preparing a report proposing the designation of *Y.e.*^{rh+} as a separate and distinct species from *Y.e.*^{rh-} and *Y. pseudotuberculosis* based on serological and deoxyribonucleic acid homology studies of *Y.e.*^{rh+} strains, in addition to a statistical analysis of the information in the present report.

As a result of the critical importance of the incubation temperature in the isolation and identification of *Y.e.*^{rh+}, it is apparent that

many infections caused by this organism have gone undetected or have been misdiagnosed. The loss of this information has, so far, placed in doubt the extent of infections caused by *Y.e.*^{rh+}, the virulence of the organism, and its epidemiology.

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