# Yersinia enterocolitica Isolates from Humans in California, 1968–1975

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This paper reports on the serological and biochemical characteristics of 24 human isolates of *Yersinia enterocolitica* submitted to the California Department of Health from 1968 through 1975. Nine different serotypes were represented. The majority of strains were serotype O:8 (six strains) and serotype O:5 (five strains). Sources of the isolates included feces (12 cases), blood (3), sputum or throat (3), bile or bowel drainage (2), wounds (2), breast abscess (1), and skin abscess (1). Clinical histories indicated a number of different syndromes. Underlying medical conditions existed in 13 cases. Results of selected biochemical tests and antimicrobial susceptibility tests on the strains indicated grouping compatible with the O serotypes of the organisms.

Cases of human infection with Yersinia enterocolitica have been reported with increasing frequency throughout the world. The majority of these cases have been reported in Europe (1, 15, 16, 19) and, in recent years, in Canada (7, 27, 28). Reports from Japan and Africa indicate the probable worldwide occurrence of this infection (2, 21, 22, 35). The organism was first recognized in the United States in New York (23), but several years intervened before further reports of isolates in this country were published (5, 24, 26).

In Europe, gastroenteritis was the most common clinical finding, followed by acute syndrome of the right iliac fossa (1, 15, 19). The majority of European isolates belong to serotype O:3, with serotype O:9 constituting the next most frequently occurring serotype (15, 19). In a survey of the occurrence of Y. enterocolitica infections in Canada, Toma and Lafleur (28) reported on 265 cultures isolated from humans in Canada; 244 were from cases of acute gastroenteritis. Serotype O:3 was the predominant serotype (217 of 256 isolates). Reports from Africa and Japan indicate the predominance of serotype O:3 in these countries. Phage typing has been used to further differentiate the organisms, particularly those of serotype O:3 (18).

In comparison with Canada and Europe, relatively few isolates have been reported from the United States, and these have been from patients with a variety of clinical symptoms. Serotype O:8 appears to be the predominant serotype, with only one reported isolation of serotype O:3 (28). In 1972, Weaver and Jordan (32) compiled a listing of 27 isolates from humans in the United States; 15 of these isolates were serotype O:8; 3 were serotype O:5; 4 were "new" serotypes, and there was one each of type O:1 or 2?, 11, 13, 15, and 16. This compilation includes 3 isolates reported previously in the literature (5, 24, 26) and 5 of the 24 isolates reported here (Table 1). The isolate designated B308, type O:1 or 2?, has since been identified as a type O:3 by T. J. Quan (personal communication) and represents the only serotype O:3 reported in the United States prior to the one reported in this paper. Recently, 3 other isolates of serotype O:8, 2 new serotypes, and 12 isolates of serotype O:17 have been reported (4, 9, 11, 20). One of the serotype O:8 isolates was from an interfamilial outbreak reported by Gutman et al. (9) which involved 16 people and led to two deaths.

In the 8-year period between 1968 and 1975, the Microbial Diseases Laboratory of the California Department of Health has received for identification or confirmation 24 strains of Y. *enterocolitica* isolated from humans by laboratories throughout the state. The results of biochemical tests and serotyping are presented here to indicate the diversity of serotypes and biochemical reactions that this organism can present in this geographic area. Results on epidemiologic investigations associated with the isolates will be reported subsequently.

### **MATERIALS AND METHODS**

Organisms included in this study were isolated from clinical specimen materials by local hospital, clinical, or public health laboratories and were submitted to the California State Microbial Diseases Reference Laboratory for confirmation or assistance in identification (Table 1). Sources of the isolates included feces (12 cases), blood (3), sputum or throat

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CSDH labo- ratory no. <sup>a</sup>	Case no.	O sero- type	Age (yr)	Sex	Source	Symptoms <sup>b</sup>	Underlying conditions	Remarks
6362-1-68	1	8	2	М	Blood	_	_	CDC no. B1023 (reference 32)
5157-1-69	2	11	69	М	Bile	_	Cholelithiasis	CDC no. B3073
1789-2-69	3	8	6	F	Throat	_	Cystic fibrosis	CDC no. B3571
<b>§22-2-70</b>	4	8	57	М	Abdominal wound infection	_	Crohn's disease, post-op colec- tomy	CDC no. B6210
4447-1-71	5	16	85	F	Sputum	AP, D, F	Hypertension	CDC no. B8455
1271-2-72	6	10	32	М	Throat	F	Burns	
3995-2-72	7	8	10	М	Blood, ile- ostomy, eye	AP, D, F	Eye trauma	
10366-2-72	8	3	43	F	Breast	Abscess	S/P mammoplasty	
13281-2-72	9	6	29	М	Feces	A <b>P</b> , D	Heroin addiction, sickle cell ane- mia SBE, artificial	
1010 1 70	10		45		D		valve 2 Diskator	
1619-1-73	10	11	45	M	Feces	AP, D	? Diabetes	
1212-2-73	11	8	Z 70	M	Feces	- F	Nono –	
0560 9 79	12	D	19		B1000	r C	Prodet consineme	
9000-2-70 6050 1 74	10	10	40	г Г	Face		Neme	
5250 1 75	14	5	41	г	Feces		None	
7896 1 75	16	5	74	M	Powel	D, AF, F	Colon cominomo	
1030-1-13	10	5	14	IVI	wound abscess	r	Colon carcinoma	
9088-1-75	17	6	26	F	Feces	_	Myasthenia gravis	Reported by Greenwood et al. (reference 8)
10757-Ent	18	5	39	F	Feces	AP, V	None	
0055-1-75	19	1	81	M	Blood	S	Anemia, diabetes	
2207-2-75 <sup>c</sup>	20	$13^d$	9	M	Feces	D	None	
2208-2-75 <sup>c</sup>	21	$13^d$	7	M	Feces	0	None	
2209-2-75 <sup>c</sup>	22	134	34	M	Feces	0	None	
9747-2-75	23	8	44	F	Skin	Abscess	None	
11358-2-75	24	5	8mo.	F	Feces	D	None	
	1	1	1	1	1	1	1	

 TABLE 1. Y. enterocolitica cases in California (1968–1975)

<sup>a</sup> CSDH, California State Department of Health.

<sup>b</sup> Abbreviations: AP, Abdominal pain; D, diarrhea; F, fever; C, cough; V, vomiting; S, sepsis. Symbols: -, Not known: 0, no symptoms.

<sup>c</sup> Members of the same family.

<sup>d</sup> Cross-reacts with antiserum to serotype O:7.

(3), bile or bowel drainage (2), wounds (2), breast abscess (1), and skin abscess (1). The clinical diagnosis is also given in Table 1. Further information regarding the clinical status of the patients and epidemiologic information will be presented in a subsequent paper.

Upon receipt of each culture, a smear was prepared, Gram stained, and examined microscopically. Plates of heart infusion agar (Difco) and heart infusion agar with 5% sheep blood were streaked for isolation. After incubation for 18 to 24 h at 35°C, the plates were examined by using a stereoscopic microscope to determine colony types and other growth characteristics. Single-colony pickings of each type of colony observed were transferred to heart infusion agar slants and to heart infusion broth. Gramstained smears of these pickings were prepared from the agar slant after 18 to 24 h of incubation at  $35^{\circ}$ C. The heart infusion broth picking was used to inoculate the various other test media used.

Organisms for this study were received over a period of 8 years. Because modified or additional techniques were used in the identification scheme for Y. enterocolitica species during this period of time, stock cultures made from the single-colony pickings of these organisms which had been stored as lyophilized cultures or kept frozen in blood on alundum granules at  $-70^{\circ}$ C were reconstituted and retested so that a better comparison of biochemical profiles of the entire group could be made. All cultures were then tested in duplicate; one set of tests was incubated at room temperature (range, 20 to

 $25^{\circ}$ C) and the other at  $35^{\circ}$ C. Readings were made at the end of 1, 2, 3, 4, and 7 days unless otherwise indicated.

Catalase tests were performed on agar-slant growth by flooding with  $H_2O_2$  (3% by volume). A 1% solution of N,N-dimethyl-*p*-phenylenediamineoxalate (6) was used to determine the oxidase reaction of growth on heart infusion agar. Indole was detected by growing the organism in 1% tryptone broth (Difco) plus 0.5% NaCl, extracting with xylene, and then adding Kovac reagent (29). Simmons citrate agar (Difco) was used to determine carbon utilization, and Christensen urea agar (Difco) was used to determine urea hydrolysis. The ability to reduce nitrates to nitrites was determined by growing the test organisms in nitrate broth (Difco) and adding the test reagents sulfanilic acid and 5-amino-2naphthalenesulfonic acid (1,6-Cleves acid) in that order. Using MR-VP medium (Difco), the Voges-Proskauer test was performed by the method of Levine et al. (13) with the addition of  $\alpha$ -naphthol. Triple sugar iron agar tests were performed as described by Edwards and Ewing (10).

Motility was observed in a semisolid medium (GI medium, Difco). Motility tests were set up in triplicate and incubated at 20°C, room temperature (RT), and 35°C. ONPG tests (10) were read after 24 h of incubation. Final readings were made after 48 h of incubation for tests for malonate utilization (10) and inhibition of growth by KCN (10). Moeller's arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase tests were read daily for 4 days (10). Utilization of acetate and tests for production of phenylalanine deaminase were determined by the methods described by Edwards and Ewing (10). Hydrolysis of esculin was determined by the method described by Sneath (25). The ability of the organisms to grow on MacConkey agar (Difco), SS agar (Difco), and cetrimide agar (14) was noted.

Production of acid from individual carbohydrates was determined by using extract broth (BBL) containing 1.0% of the specific carbohydrate along with Andrade indicator. Production of gas on selected carbohydrates was detected by use of Durham tubes.

Serological typing of the organisms was done by slide agglutination tests using antisera prepared in this laboratory to serotypes O:1 through O:21. Antisera were prepared to stock strains of Y. enterocolitica obtained from S. Winblad using techniques described by him (33). The antisera were absorbed where indicated. Antigens for the slide test consisted of heavy suspensions of the organisms in 0.5%phenolized saline prepared from cultures on brain heart infusion agar which had been incubated for 24 to 48 h at  $22^{\circ}$ C.

Tests for antibodies in the patient's sera were performed by the tube agglutination procedure using a 30-min incubation at 37°C followed by centrifugation at 800  $\times g$  for 7 min. Antigens for this test were prepared from the patient's isolate and from stock strains by the same method used for serotyping antigens, with the exception that antigens for the tube agglutination procedure were washed and standardized to a no. 2 McFarland turbidity standard. Antigens for the detection of agglutinins in the patients' sera were therefore OH antigens rather than O antigens used in the preparation of serotyping antisera.

Antimicrobial susceptibility tests were performed by the Kirby-Bauer technique (3) at  $35^{\circ}$ C incubation.

## RESULTS

Table 2 indicates the biochemical reaction of 24 strains of Y. enterocolitica isolated from humans in California over an 8-year period. All cultures produced catalase and urease and were negative for oxidase, gelatin hydrolysis, lysine decarboxylase, arginine dihydrolase, phenylalanine deaminase, and utilization of citrate. All cultures, with the exception of no. 5157-1-69 and no. 1619-1-73, produced acid slants and acid butts in triple sugar iron agar after 24 h of incubation at 35°C. The majority of these cultures produced slight amounts of gas between days 2 and 7 of incubation. None of the strains produced  $H_2S$  in triple sugar iron agar tests. Cultures no. 5157-1-69 and no. 1619-1-73 produced alkaline slants and acid butts in triple sugar iron agar. These two cultures were the only strains which failed to ferment sucrose. Both of these isolates were serotype O:11.

Acid was produced by all cultures at RT and 35°C incubation from the following carbohydrates: glucose, maltose, cellobiose, mannitol, sorbitol, trehalose, and arabinose. None of the cultures fermented adonitol, dulcitol, melibiose, erythritol, or  $\alpha$ -methyl glucoside. Durham tubes for the detection of gas production were included in the following carbohydrates: glucose, lactose, maltose, sucrose, adonitol, glycerol, inositol, and cellobiose. The majority of cultures produced small amounts of gas, usually after 48 h of incubation at RT, from glucose, maltose, sucrose, glycerol, and cellobiose. All cultures were motile at room temperature, but motility results varied when tests were incubated at 20 and 35°C.

The results of selected biochemical reactions of the 24 strains grouped by serotype are presented in Table 3. Nine serotypes are represented; serotype O:8 (six strains) and serotype O:5 (five strains) were the most frequently isolated. All strains of serotype O:6, O:10, O:13, and O:16 and an aberrant strain (no. 1789-2-69) of serotype O:8 produced indole, hydrolyzed esculin, and fermented salicin. Strains of serotype O:1 and O:3 were negative for indole, esculin, and salicin, whereas type O:5 and type O:11 strains were negative or late indole producers. Serotype O:8 strains were indole positive and, with the exception of no. 1789-2-69, failed to hydrolyze esculin or ferment salicin during incubation at room temperature.

Antimicrobial test results also differed ac-

	Temp							
<b>Biochemical reaction</b>		R Ta			35°C			
	+0	(+)	-	+	(+)	-		
Motility <sup>c</sup>	234	1	0	5	3	16		
Catalase	24	0	0	24	0	0		
Oxidase	0	0	24	0	0	24		
Nitrate to nitrite	23	0	1	23	0	1		
Gelatin	0	0	24	0	0	24		
Citrate (Simmons)	0	0	24	0	0	24		
Indole	14	2	8	14	4	6		
Urease	24	0	0	20	4	0		
Voges-Proskauer	22	0	2	9	6	9		
Esculin hydrolysis	9	0	15	9	0	15		
Beta-galactosidase (ONPG)	24	0	0	23	0	1		
Malonate	6		18	0		24		
Phenylalanine deam-	0	0	24	0	0	24		
Sodium occtoto	16	-	1	E	9	10		
Sodium acetate	10			0	3	10		
Lysine decarboxylase	0	0	24		0	24		
Arginine dinydrolase		0	24		0	24		
Ornithine decarbox-	24	0	0	23	0	1		
KCN	3		21	2		22		
Growth on:								
MacConkey	24	0	0	24	0	0		
SS	18	ĥ	ň	24	Ő	ŏ		
Cetrimide	9	5	10	27	4	18		
Glucose			10	-	7	10		
Acid	24	0	0	24	0	0		
Gas	15		2	4	i	10		
Y vlose	23			22		19		
	20	U	1	20	U	1		
Lactose	2	1	21	2	1	21		
Maltose								
Acid	24	0	0	23	1	0		
Gas	8	10	6	17	5	2		
Sucrose								
Acid	22	0	2	22	0	2		
Gas	8	11	5	11	0	13		
Raffinose	1	1	22	3	0	21		
Adonitol	0	0	24	0	0	24		
Dulcitol	0	Ō	24	Ŏ	Ó	24		
Glycerol <sup>e</sup>								
Acid	23	0	1	23	1	0		
Gas	15	7	2	20	0	4		
Inositol			-			-		
Acid	3	19	2	12	6	6		
Gas	0	0	24	5	3	16		
Mannitol	24	0	0	24	0	0		
Sorbitol	24	0	0	24	0	0		
Salicin	9	0	15	10	1	13		
Trehalose	24	0	0	24	0	0		
Cellobiose <sup>e</sup>								
Acid	23	1	0	23	1	0		
Gas	8	14	2	17	1	6		

 TABLE 2. Biochemical reactions of 24 strains of Y.

 enterocolitica incubated at two temperatures

TABLE 2-Continued

		Temp							
<b>Biochemical reaction</b>		R T <sup>a</sup>		35°C					
	+0	(+)	-	+	(+)	-			
Rhamnose	1	0	23	1	0	23			
Melibiose	0	0	24	0	0	24			
Arabinose	23	1	0	24	0	0			
Erythritol	0	0	24	0	0	24			
$\alpha$ -Methyl glucoside	0	0	24	0	0	24			

<sup>a</sup> Room temperature, 20 to 25°C.

<sup>b</sup> Symbols: +, Positive within 1 to 2 days; (+), positive within 3 to 7 days; -, negative.

<sup>c</sup> Motility tests were incubated at 20°C and gave the following results: 12+, 4(+), and 8-.

<sup>d</sup> Number of strains.

<sup>e</sup> Durham tubes added to detect gas production.

cording to serotype (Table 4). All serotype O:8 strains, with the exception of no. 1789-2-69, were sensitive to ampicillin, carbenicillin, and cephalothin. The two strains of serotype O:11 organisms were resistant to cephalothin only, whereas the remaining serotypes, with four exceptions (Table 4), were resistant to ampicillin, carbenicillin, and cephalothin. All strains were sensitive to chloramphenicol, gentamicin, kanamycin, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline.

The results of tube agglutination tests on patients' sera tested against an OH antigen prepared from their own isolate are given in Table 5. Sera were obtained from 10 of the 24 patients. In some cases, only one serum specimen was obtained; in others, multiple samples drawn over a period of time were available. In 5 of the 10, antibodies against the isolate were demonstrated. In all cases where agglutinins were present, titers of at least 1:160 were obtained on one or more specimens.

### DISCUSSION

Nine different O serotypes are represented in the 24 cases reported here. Serotype O:8 (six cases) and O:5 (five cases) were the most frequently isolated strains, with serotypes O:1, O:3, O:6, O:10, O:11, O:13, and O:16 represented by three or fewer strains. The isolates exhibited differences in biochemical reactions, especially in tests for indole production, hydrolysis of esculin, and fermentation of salicin. Of the 24 strains, 16 produced indole at RT and at  $35^{\circ}$ C, 14 of them within 24 h of incubation. Nine of the 16 produced positive reactions in tests for hydrolysis of esculin and fermentation of salicin. These differences were related to the O serotype of the strains.

With few exceptions, reports in the literature

O sero-	CSDH no.ª	Indole (RT)	Escu-	Salicin (BT)	Gas from	Lactose (RT)	Su-	Gas fr cro	om su- ose	Biotype (Nilehn)	Other
type		()		(101)	(RT)		crose	RT	35°C	(Tunenii)	
Type 1	6959-1-74	-	-	-	+	-	Α	-	+	3	+ KCN (RT)
<b>—</b>	0055-1-75	-	-	-	(+)	-	A	(+)	+	3	
Туре 3	10366-2-72	-	-	-	-	-	A	(+)	-	4	<ul> <li>Xylose</li> <li>Ornithine</li> <li>Decarboxylase</li> </ul>
Type 5	3214-2-73	-	-		(+)	-	A	+	-	2	(+) Indole (35°C)
	5350-1-75	-	-	-	+	- 1	A	+	+	2	(+) Indole (35°C)
	7836-1-75	-	- 1	-	+	-	A	(+)	_	3	
	10757-Ent.	-	-	- 1	(+)	-	A	(+)	+	3	
	11358-2-75	-	-	-	+	-	A	-	-	2	(+) Indole (35°C)
Type 6	13281-2-72	+	+	A	+	-	A	(+)	+	1	
	9088-1-75	+	+	A	+	-	A	(+)	+	1	
Туре 8	6362-1-68	+	-	-	+	-	A	+	-	2	<ul> <li>Sodium acetate (RT)</li> </ul>
	1789-2-69	+	+	A	_	A	Α	-	-	1	A Raffinose + KCN (RT) - nitrate
	922-2-70	+	_	_	(+)	_	A	(+)	_	2	(A) Salicin (35°C)
	3995-2-72	+	_	-	(+)	_	A	+	+	2	
	1212-2-73	+	_	- 1	+	(A)	Ā	(+)	+	2	– Lactose (35°C)
	9747-2-75	+	_	_	+	_	A	+	+	2	
Type 10	1271-2-72	+	+	Α	(+)	A	Ā	(+)	_	1	
<b>VP</b>	9560-2-73	+	+	Ā	+	_	Ā	+	_	1	
Type 11	5157-1-69	(+)	_	-	(+)	-	_	-	-	2	+ KCN (RT) - VP
	1619-1-73	(+)	-	-	+	-	_	-	_	2	A Salicin(35°C) + KCN (35°C) - VP
Type 13	2207-2-75	+	+	A	+	-	Α	+	+	1	
	2208-2-75	+	+	A	+	-	Α	+	_	1	
	2209-2-75	+	+	A	+	-	Α	(+)	_	1	
Type 16	4447-1-71	+	+	A	+	-	Α	(+)	+	1	+ KCN (35°C) A Rhamnose (A) Lactose (35°C)

TABLE 3. Selected biochemical reactions of 24 strains of Y. enterocolitica by serotype

<sup>a</sup> CSDH, California State Department of Health.

<sup>b</sup> When the temperature of incubation is not indicated, the stated reaction was obtained at both RT and  $35^{\circ}$ C. Symbols: +, Positive within 1 to 2 days; (+), positive within 3 to 7 days; -, negative. Abbreviations: A, Acid within 1 to 2 days; (A), acid within 3 to 7 days; RT, incubation at room temperature (range, 20 to  $25^{\circ}$ C);  $35^{\circ}$ C, incubation at  $35^{\circ}$ C.

indicate that Y. enterocolitica strains are motile at 22°C but are nonmotile at 35 to 37°C. Delorme et al. (7) indicated that 1 isolate of 35 studied was motile at 37°C, and Braunstein et al. (5) reported a strain of Y. enterocolitica isolated from a case of mesenteric lymphadenitis that developed motility "slowly" at 37°C. All of the strains reported here were motile at RT incubation. Two of the strains were motile at 35°C when tests were first performed upon arrival of the cultures in this laboratory; one strain was motile after 24 h of incubation at 35°C, and the other was weakly motile after 48 h of incubation. However, when tests were repeated on the 24 strains removed from frozen storage, 8 of the 24 were motile after 48 h or more of incubation at  $35^{\circ}$ C. It is possible that the motility of the organism at  $35^{\circ}$ C was enhanced by the number of transfers that occurred before the organisms were stored in the frozen state. Further studies on the effect of transfer and incubation temperature utilizing techniques such as flagella stain, phase contrast, and electron microscopy will be necessary to clarify the nature of our results. Although there are relatively few exceptions to the general rule of motility at 22 to  $25^{\circ}$ C but lack of motility at 35 to  $37^{\circ}$ C, on occasion these organisms may be motile at 35 to  $37^{\circ}$ C. Therefore, the possibility of this type of reaction should be considered when screening primary isolates.

The taxonomy of Y. enterocolitica organisms

O sero- type		CSDH no.	Ampi- cillin	Car- benicil- lin	Cepha- lothin	Strep- tomy- cin
Туре	1	6959-1-74	R	R	R	s
•••		0055-1-75	R	R	R	S
Type 3	3	10366-2-72	R	R	R	S
Type	5	3214-2-73	Ι	S	R	S
•••		5350-1-75	R	R	R	S
		7836-1-75	R	R	R	R
		10757-1-75	R	R	R	S
		11358-2-75	R	S	R	$\mathbf{S}$
Type	6	13281-2-72	R	R	R	S
•••		9088-1-75	R	Ι	R	S
Type 3	8	6362-1-68	S	S	S	S
••		1789-2-69	R	R	R	$\mathbf{S}$
		922-2-70	S	S	S	$\mathbf{S}$
		3995-2-72	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{s}$
		1212-2-73	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{s}$
		9747-2-75	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{S}$	$\mathbf{S}$
Type	10	1271-2-72	R	R	R	$\mathbf{S}$
		9560-2-73	$\mathbf{S}$	$\mathbf{S}$	R	$\mathbf{S}$
Type	11	5157-1-67	$\mathbf{S}$	$\mathbf{S}$	R	$\mathbf{S}$
		1619-1-73	$\mathbf{S}$	$\mathbf{S}$	R	$\mathbf{S}$
Туре	13	2207 - 2 - 75	R	R	R	$\mathbf{S}$
		2208-2-75	R	R	R	$\mathbf{S}$
		2209-2-75	R	R	R	$\mathbf{S}$
Туре	16	4447-1-71	R	R	R	S

 TABLE 4. Antimicrobial susceptibility test results on

 24 strains of Y. enterocolitica<sup>a</sup>

<sup>a</sup> Abbreviations: CSDH, California State Department of Health; R, resistant; S, sensitive; I, intermediate.

continues to be a subject of debate among investigators in this field (12, 34). The genus is now included in the family Enterobacteriaceae (17). It has been suggested that not all organisms currently labeled as Y. enterocolitica are members of the same species because of the differences in biochemical and antigenic characteristics (12, 34). Winblad (personal communication) has indicated that a separation of Y. enterocolitica (serotypes 1-3, 8, 9, 11, and 12) and Y. enterocolitica-like organisms (serotypes that exhibit positive reactions in indole, esculin, and salicin tests) may be appropriate. Tables of biochemical reactions currently appearing in the literature have been primarily based upon human isolates of serotypes O:3 and O:9 with relatively few representatives of other serotypes. Wauters and colleagues (30, 31) have studied the O and H antigens of several strains of Y. enterocolitica and have extended Winblad's original serotypes to 34 O serotypes, with the division of group O:5 into two subgroups based upon H antigens and biochemical characteristics.

The difficulties encountered in isolation of Y. enterocolitica from specimens exhibiting a mixed flora have been commented upon by several investigators (4, 7, 8, 15, 19, 27). These difficulties coupled with the lack of knowledge of the source of human infection may be respon-

 TABLE 5. Results of tube agglutination tests on patients' sera using OH antigens prepared from patients own isolate

O serotype	CSDH no. <sup>a</sup>	Source of isolete	Date of onset	Date of isolate	Date serum taken	Tube ag- glutination titer <sup>o</sup>
Type 3	10366-2-72	Breast	9-23-72	9-28-72	12-6-72	_c
Type 5	3214-2-73	Blood	6-73	8-13-73	9-24-73	320
••					12-17-73	320
	5350-1-75	Feces	11-14-74	2-26-75	4-9-75	
	10575-Ent	Feces	12-8-74	6-12-75	11-13-74	_
					6-18-75	
Type 6	13281-2-72	Feces	Unknown	12-3-72	10-11-72	160
51					10-24-72	80
					12-11-72	80
					12-28-72	80
					4-5-73	160
Type 8	3995-2-72	Eye	7-23-72	7-24-72	8-3-72	160
51		•			9-14-72	1280
					10-3-72	1280
	1212-2-73	Feces	7-3-73	$\sim$ 7-10-73	7-17-73	320
					8-1-73	640
					9-12-73	320
					1-4-75	40
Type 10	1271-2-72	Throat	7-72	7-12-72	8-17-72	_
01	9560-2-73	Sputum	11-73	12-1-73	12-10-73	1280
		•			2-13-74	160
Type 11	1619-1-73	Feces	Chronic	1-21-73	4-3-72	_
					2-23-73	_

<sup>a</sup> CSDH, California State Department of Health.

<sup>b</sup> Reciprocal of highest dilution (final) of patient's serum giving 50% or greater agglutination of antigen.

<sup>c</sup> No reaction at lowest dilution of patient's serum (1:20).

## Vol. 4, 1976

sible for the small numbers of isolates of Y. enterocolitica encountered in this country. The results of biochemical and serologic tests performed on the isolates reported here should add to the information regarding geographic distribution of serotypes and to data regarding the diversity of biochemical reactions that may be encountered.

With the isolation of further serotypes from man and his environment, the clarification of the taxonomy and use of a definitive antigenic classification becomes even more important to aid laboratory personnel in the identification of these organisms and epidemiologists in their attempt to describe the source and nature of this infection in man.

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