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Clinical Isolation of *Yersinia enterocolitica*: Cold Temperature Enrichment

J. R. GREENWOOD, S. M. FLANIGAN, M. J. PICKETT,* AND W. J. MARTIN

Department of Bacteriology, University of California and Microbiology Section, Clinical Laboratories, University of California at Los Angeles Hospital and Clinics, Los Angeles, California 90024*

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A cold-temperature enrichment procedure was used to isolate *Yersinia enterocolitica* serotype 6 from a clinical stool specimen. The use of conventional laboratory media and enrichment procedures failed to isolate this organism.

Yersinia enterocolitica can produce human disease with a variety of clinical syndromes. These include abscesses of the spleen and colon, gastroenteritis, bacteremia, peritonitis, cholecystitis, and mesenteric lymphadenitis (4). Septicemia with a 50% mortality rate has been reported in the compromised host (3). Because of the important consequences in this group of patients, proper antimicrobial therapy must be started promptly. Unfortunately, *Y. enterocolitica* is not easily isolated and identified in the clinical laboratory. Because of the slow growth of this organism at 37 C and biochemical reactions that superficially resemble those of other members of the family *Enterobacteriaceae*, it can be frequently overlooked (1). We report the use of a low-temperature enrichment procedure to isolate an unusual serotype of *Y. enterocolitica* from a fecal specimen of an immunosuppressed patient.

This strain of *Y. enterocolitica* was cultured from a 26-year-old female who was admitted to the University of California at Los Angeles Hospital and Clinics with the chief complaint of breathing difficulty secondary to myasthenia gravis. In 1971, the patient underwent a thymectomy, and in 1973 she had reexploration to determine if there was any residual thymus; none was found. Initially she was given steroid medication every other day that consisted of Medrol, 100 mg. During the course of her hospitalization this was changed to Decadron, 20 mg, on the same schedule and then to prednisone, 80 mg, each morning. Four weeks from the date of admission, the patient was noted to be doing well, but diarrhea developed. At this time a stool specimen was submitted to the clinical laboratory for culture and parasite examination.

The University of California at Los Angeles Clinical Microbiology Laboratory processed this specimen according to their routine procedure for stools: (i) direct inoculation of primary MacConkey, XLD, and Hektoen enteric agar plates and GN enrichment broth, and (ii) the following morning, subculture to secondary MacConkey, XLD, and Hektoen enteric plates from the 14- to 18-h GN broth. All plates were incubated 48 h at 37 C before being discarded as negative. The Clinical Microbiology Laboratory reported no enteric pathogens recovered from this specimen.

At the same time the above-mentioned media

TABLE 1. *Biochemical characteristics and antiobiogram of Yersinia enterocolitica serotype 6 isolate*

Test or substrate[a]	Result	Antibiotic	Result[b]
KIA		Gentamicin	S
Slant	Alkaline	Kanamycin	S
Butt	Acid	Chloramphenicol	S
H_2S	−	Streptomycin	S
Urease	+	Carbenicillin	R
β-Galactosidase (ONPG)	+	Gantrisin	S
Lysine decarboxylase	−	Cephalothin	R
Ornithine decarboxylase	+	Polymyxin B	S
Motility		Ampicillin	I
37 C	−		
22 C	+		
Citrate (Simmons)	−		
Voges-Proskauer			
37 C	−		
22 C	+		
Indole	+		
Methyl red			
37 C	+		
22 C	+		
Glucose	+		
Lactose	−		
Sucrose	+		
Mannitol	+		

* KIA, Kligler iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b] Kirby-Bauer method (2). Abbreviations: S, sensitive; R, resistant; I, intermediate.

were inoculated, 1 loopful of stool specimen was placed in 10 ml of buffered saline. This tube was then placed in a refrigerator (4 C) and then subcultured to SS agar (Difco) at 10 and 21 days (5). The SS agar plates were incubated at room temperature for 48 h before they were examined for colonies typical of *Y. enterocolitica*. This enrichment procedure (S. Toma and R. Deidrick, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C42, p. 34) was instituted by us to determine the incidence of *Y. enterocolitica* in stool specimens submitted to the clinical laboratory (manuscript in preparation).

The 10-day subculture from the buffered saline revealed numerous lactose-negative colonies. Several of these colonies were picked, but none of them was *Y. enterocolitica*. The 21-day subculture again revealed numerous lactose-negative colonies, several of which were *Y. enterocolitica* serotype 6. The biochemical characteristics and antibiogram of this isolate are given in Table 1.

Since no classical enteric pathogens were isolated, *Y. enterocolitica* appears to be the etiologic agent of the diarrhea exhibited by this patient. Although the diarrhea was short termed and self-limited, the significant point is that conventional laboratory media and procedures failed to recover the organism. This is even more important when one considers the immunosuppressed nature of this host and the propensity of this organism to produce septicemia. Efforts are now underway in our laboratories to develop a more rapid and therefore more clinically useful enrichment broth for *Y. enterocolitica*.

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