Unusual \textit{Yersinia enterocolitica} Isolates Not Associated with Mesenteric Lymphadenitis

EDWARD J. BOTTON, BRENT CHESTER, MOISES S. MALOWANY, AND JONA ALLERHAND

Department of Microbiology, The Mount Sinai Hospital, New York, New York and Department of Pathology, Mt. Sinai Services Unit, City Hospital Center at Elmhurst, Elmhurst, Queens, New York

Received for publication 28 January 1974

Thirteen \textit{Yersinia enterocolitica} were recovered from a variety of clinical sources. Of these, only one was associated with mesenteric lymphadenitis and belonged to serotype 8. The 12 remaining strains were isolated from nonmesenteric sources and belonged to serotype 17. All strains exhibited the main characteristics of \textit{Y. enterocolitica} which differentiated them from other \textit{Enterobacteriaceae}, i.e., motility at 22 C but not at 37 C, positive urease and ornithine decarboxylase activities, and negative phenylalanine deaminase. These 12 strains differed, however, from other \textit{Y. enterocolitica} previously described in the United States in that they fermented rhamnose and raffinose at 22 C, and failed to grow on Salmonella-Shigella and Hektoen-Enteric agars.

\textit{Yersinia enterocolitica}, a small gram-negative coccobacillus which culturally and biochemically resembles other members of the family \textit{Enterobacteriaceae} (2), is capable of causing a variety of infections both in animals and humans. These may appear as gastroenteritis, septicemia, acute polyarthritis, and erythema nodosum (1). This microorganism, along with Pasteurella (\textit{Yersinia}) \textit{pseudotuberculosis}, is frequently associated with acute mesenteric lymphadenitis and terminal ileitis closely resembling appendicitis. This association has been frequently documented by European workers (6, 10, 11, 21), and of 150 human isolates studied by Nilehn in Sweden (12), 148 were cultured from enteric contents of patients most of whom presented symptomatology suggesting acute appendicitis. The two remaining strains were recovered from patients with chronic lymphocytic leukemia, one from a mesenteric lymph node and one from blood.

Although the first \textit{Y. enterocolitica} infections were reported in the United States in 1939 (13), recently more than 1,000 new cases have been described in other parts of the world, Europe in particular, whereas in the United States additional reports have been infrequent. Of five recent reports, three documented the mesenteric lymphadenitis association (3, 19, 20), the fourth an interfamilial outbreak of enteritis (5), and the fifth, reported by Sonnenwirth (16), describes \textit{Y. enterocolitica} as a causative agent of meningitis.

During a 2-year period (1971-1973) in our laboratories, 13 \textit{Y. enterocolitica} isolates were recovered from a variety of clinical sources, 12 of which were not associated with mesenteric lymphadenitis. These 12 isolates, although classified undoubtedly as \textit{Y. enterocolitica}, differed in biochemical, cultural, and serological characteristics from strains previously reported in the United States.

The aim of this report is to describe these unusual \textit{Y. enterocolitica}, which may be more widespread in this country than is presently appreciated.

**MATERIALS AND METHODS**

Table 1 shows the sources of the 13 \textit{Y. enterocolitica} isolates. These strains were recovered from clinical specimens submitted to the Diagnostic Microbiology Laboratories of The Mount Sinai Hospital and City Hospital Center at Elmhurst.

Microscopic morphology was studied by gram-stained smears of colonies developing on 5% sheep blood agar after 24 h of incubation at 37 C. Motility was determined by phase microscopy (×40) examination of hanging-drop preparations of overnight glucose broth cultures incubated at 22 and 37 C, respectively. Growth characteristics were studied on 5% sheep blood agar (BBL), MacConkey agar, Hektoen-Enteric agar (H-E), Endo and Salmonella-Shigella agars (SS, Difco), and on xylose-lysine deoxycholate agar (XLD, Scott Laboratories, N.Y.) at 22 and 37 C for 24 and 48 h of incubation.

Biochemical tests were performed at 22 and 37 C as outlined by Edwards and Ewing (4). Peptone-agar containing 1% (wt/vol) concentrations of various carbohydrates with Andrades indicator were used for fermentation studies. For the optimal detection of indole production, several methods and media were employed: (i) tryptone broth (Difco), (ii) SIM media
(BBL), and (iii) the spot-test method (7), utilizing the colonies from sheep blood agar incubated at 22 and 37 C. Ehrlich’s or Kovac’s reagents, or both, were used as indicators. Tryptone broth and SIM were inoculated in duplicate and tested after overnight incubation at 37 C and after 48 h at 22 C. Urease activity was studied on Christensen’s urea agar (Difco) and with Rustigan’s urea broth (BBL). Tests for beta-galactosidase activity were performed at 22 and 37 C by using tablets containing O-nitrophenol-beta-D-galactopyranoside (ONPG, Key Scientific Products Co., Los Angeles, Calif.). For oxidase tests, oxidase disks (Difco) were utilized, and for testing for extracellular deoxyribonuclease (DNase), DNase agar (Difco) was used.

Serological typing of eight of the strains was performed through the courtesy of S. Winblad of Lunds University, Sweden.

Antibiotic susceptibility tests were performed by the two-tube broth dilution method of Schneierson and Amsterdam (14).

RESULTS

All Yersinia strains were gram-negative coc-cobacilli displaying some tendency toward pleor-
all strains by suspending lead acetate strips into the tubes. With the exception of the lymph node isolate, biochemically, all strains were similar in their fermentative capability with regard to several carbohydrates. The lymph node isolate failed to ferment esculin, raffinose, rhamnose, and salicin, whereas the nonmesenteric isolates all utilized these substrates. At 22 C, raffinose and rhamnose were fermented within 24 h, whereas 72 to 120 h were required when these substrates were incubated at 37 C. None of the strains fermented dulcitol or lactose after 7 days of incubation, but all gave a rapid positive ONPG reaction indicative of β-galactosidase activity.

With regard to indole production, the lymph node isolate gave an immediate strong reaction at 22 and 37 C, with all three procedures utilized. Of the remaining isolates, negative or weak positive reactions were observed in tryptone broth after 24 h of incubation, particularly at 37 C. However, utilizing SIM and the spot-test technique, all isolates gave a prompt and distinct indole-positive reaction at both incubation temperatures. Of the 13 isolates studied, the 12 nonmesenteric isolates were capable of growth on Simon’s citrate agar producing an alkalization of the medium after 48 h of incubation at 22 C. On Christensen’s urea agar, all strains showed signs of urea hydrolysis within 5 h of incubation which became more pronounced after 18 h, whereas in Rustigan’s urea broth, urease activity was not observed. Ornithine decarboxylase was uniformly present, but absent were lysine decarboxylase, arginine dihydrolase, oxidase, extracellular DNase, and phenylalanine deaminase activities.

Serologically, according to S. Winblad, the lymph node isolate belonged to serotype 8, and of the eight nonmesenteric strains submitted, all were designated as serotype 17. The remaining four nonmesenteric isolates all agglutinated with type 17 antisera prepared in rabbits in our laboratory. None of the type 17 isolates were agglutinated by heterologous type 3 or type 8 antisera.

Table 3 shows the antibiotic susceptibility patterns of the 13 isolates as determined by the two-tube broth dilution method. With the exception of one strain which was sensitive to ampicillin, all of the *Yersinia* strains were uniformly resistant to ampicillin, cephalothin, and penicillin, moderately resistant or resistant to tetracycline, and susceptible to chloramphenicol, gentamicin, and neomycin. Twelve of the 13 strains were susceptible to colymycin.

**DISCUSSION**

As shown in this study, of 13 *Y. enterocolitica* isolates, 1 was associated with mesenteric lymphadenitis, and 12 were derived from nonmesenteric sources. The single mesenteric lymph node isolate was serotype 8. This strain, in accordance with previous reports of *Y. enterocolitica* recovered from mesenteric lymphadenitis (3, 15, 18, 19), grew well on Endo, H-E, MacConkey, and XLD agars, and failed to ferment esculin, raffinose, rhamnose, and salicin even after incubation at 22 or 37 C for 1 week. The 12 nonmesenteric isolates belonged to serotype 17. All possessed the salient features of *Y. enterocolitica*, i.e., urease production on Christensen’s urea agar, negative phenylalanine deaminase, motility at 22 C but not at 37 C, and ornithine decarboxylase activity. In contrast to the lymph node isolate, however, these 12 strains failed to grow or grew poorly on H-E and XLD agars and fermented esculin, raffinose, rhamnose, and salicin, and in addition were slowly able to utilize sodium citrate as a sole carbon source. Regarding raffinose and rhamnose fermentation, all serotype 17 isolates fermented these carbohydrates promptly within 24 h when incubated at 22 C. At 37 C, fermentation was delayed, requiring from 48 to 120 h of incubation. These results dramatically reflect the influence of temperature on several features of *Yersinia*, namely, fermentative capability, motility, and growth potential on various media. The growth characteristics, biochemistry, and serology of these unusual *Y. enterocolitica* markedly distinguished them from previously described isolates both in the United States (3, 5, 15, 16, 19) and in Europe (1, 12, 17, 18). In these reports, of a total of 506 strains studied from human and animal sources, none were serotype 17. The majority of the isolates were serotype 3, 8, or 9, which according to S. Winblad (personal communication) are the most common *Y. enterocolitica* recovered.

Indole production in *Y. enterocolitica* is described as being either negative or positive (1, 12, 16). However, among the negative strains

| Antibiotic       | Concentration (μg/ml) | No. sensitive/no. tested |
|------------------|-----------------------|-------------------------|
| Ampicillin       | 5, 10                 | 1/13                    |
| Cephalothin      | 7.5, 15               | 0/13                    |
| Chloramphenicol  | 7.5, 15               | 13/13                   |
| Colymycin        | 2, 5                  | 12/13                   |
| Gentamicin       | 5, 10                 | 13/13                   |
| Neomycin         | 10, 20                | 13/13                   |
| Penicillin       | 2, 4 (U)              | 0/13                    |
| Tetracycline     | 2, 4                  | 0/13                    |

**Table 3. Antibiotic susceptibility of the 13 *Y. enterocolitica* isolates as determined by the modified two-tube broth dilution method.**
described, a degree of variability may exist depending upon the methodology. In the present study, only the lymph node isolate gave a distinct indole-positive reaction in tryptone broth after 24 h of incubation at 22 and 37 C. The 12 nonmesenteric isolates gave a negative or a weakly positive reaction when tested under the same conditions. All 13 isolates gave a strong indole positive reaction at both incubation temperatures when assayed in SIM medium or by the spot-test technique. It is therefore conceivable that some indole-negative Yersinia, as observed predominantly by European investigators, may be due to the use of tryptone broth in these laboratories for determining indole production (1, 12).

The exclusive recovery of Y. enterocolitica serotype 17 from a variety of clinical specimens does suggest a reservoir for this organism in the community served by The Mt. Sinai Hospital. To date, epidemiological studies have not been undertaken to uncover such a reservoir. Nevertheless, Lassen (9) in Norway recently reported the recovery of Y. enterocolitica serotype 17 from untreated drinking water.

Human infections caused by Y. enterocolitica are being increasingly appreciated by both clinicians and microbiologists. Some of the factors contributing to the previous lack of recognition of infections caused by this microorganism in the United States may be attributed to (i) the difficulty in the isolation and differentiation of Y. enterocolitica from other members of the family Enterobacteriaceae, especially when Yersinia is present in mixed bacterial population, e.g., feces; (ii) the lack of awareness of the association of Yersinia with diseases other than mesenteric lymphadenitis; and (iii) the difficulty in the identification of an unusual isolate that differs in several cultural and biochemical characteristics from more typical strains reported in the United States. The recovery of a gram-negative, nonlactose-fermenting rod which upon biochemical screening utilizing triple sugar or Kligler’s iron agar resembles a “urease positive E. coli” or a Proteus morganii should be further examined for identification as Y. enterocolitica.

ACKNOWLEDGMENTS

We wish to express our gratitude to A. C. Sonnenwirth, R. E. Weaver, and M. Weisburd for confirmation of several of the Y. enterocolitica isolates, and to S. Winblad for serological typing. We also acknowledge the encouragement of W. Mautner and S. S. Schneierson, and the technical assistance of J. Kittick, I. Kolman, and S. Schmucker.

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