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Evaluation of the Minitek and API 20E Systems for Identification of *Yersinia enterocolitica*

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ABSTRACT

The basic 12-disk Minitek system (14 tests) and the API 20E system were evaluated for identification of *Yersinia enterocolitica* strains isolated at various locations in the world. The API 20E system correctly identified all the *Y. enterocolitica* strains while the Minitek system identified 80% (20/25) of the strains. Four of the five cultures misidentified by Minitek were atypical strains isolated from vacuum-packaged meats. These four cultures were identified as *Citrobacter freundii* by Minitek due to a rhamnose-positive reaction. Minitek and API 20E are rapid and convenient systems which could prove to be beneficial for identifying *Y. enterocolitica* strains.

The awareness of *Yersinia enterocolitica* as a human pathogen has increased in the last 5 to 10 years. Extensive research has been done in several European countries (*25*), Japan (*1,31,32*), and Canada (*30*), but the seriousness of *Y. enterocolitica* infection in the United States has been ascertained only recently. *Y. enterocolitica* has been isolated from human as well as nonhuman sources. From 1968-1975, Bissett (*2*) obtained 24 human isolates of *Y. enterocolitica* in which the major clinical symptoms of the patients consisted of abdominal pain, diarrhea and fever. *Y. enterocolitica* has been isolated from water in the United States (*15*) and Europe (*19,21*). Serotype 0:17, the same serotype isolated from non-mesenteric clinical samples (*4*), has been isolated in a survey of vacuum packaged fresh beef (*13*).

Y. enterocolitica has been isolated from various other foods such as lamb, ice cream, and raw milk (*13,24,26*) and animals including the swine, canine, rodent and cow (*17,18,29*). Because similar serotypes of *Y. enterocolitica* have been isolated from humans, animals and foods, a mode of transmission of *Y. enterocolitica* to humans from contaminated foods or infected animals could be possible (*3,22*). In 1976, the first reported outbreak of foodborne illness in the United States implicating *Y. enterocolitica* occurred among school children in Oneida County, New York (*23*). Chocolate milk was determined to be the common vector of transmission in this outbreak. Serotype 0:8 was isolated from children and chocolate milk.

Rapid and reliable identification of *Y. enterocolitica* is of importance to the clinical, food or environmental microbiologist. The API 20E and Minitek systems have proven to be extremely valuable for rapidly identifying members of the *Enterobacteriaceae* family isolated from clinical samples (*14,16,20,28*). Recently, the applicability and accuracy of both miniaturized systems have been evaluated for identifying members of the *Enterobacteriaceae* isolated from foods. By using comminuted beef, pork or turkey, Guthertz and Okoluk (*12*) showed that the API 20E and Minitek systems correctly identified 96.1 and 78.3%, respectively, of the enteric strains. Further, Cox and Mercuri (*6*) demonstrated that the API 20E correctly classified 82% of the *Enterobacteriaceae* strains isolated from various foods and food products. However, for *Y. enterocolitica*, a member of the *Enterobacteriaceae*, there are insufficient data to determine the feasability of using Minitek or API 20E systems for its identification from clinical or food samples. The present study was done to determine the accuracy of the basic 12-disk Minitek and API 20E systems for identifying typical and atypical strains of *Y. enterocolitica*.

MATERIALS AND METHODS

Bacterial strains

The antigen type, origin and contributor of the 25 *Y. enterocolitica* strains used in this study are listed in Table 1. Stock cultures were maintained on Difco brain heart infusion (BHI) slants and stored at 4 C.

Conventional methods

Each strain was inoculated into 10 ml of BHI broth and incubated for 24 h at 25 and 37 C. After incubation, the broths were centrifuged and the pellet was resuspended in 10 ml of sterile distilled water. The washed cells were refrigerated at 4 C for no longer than 1 h until use. All the strains were inoculated into the following media: 1% tryptone broth, motility-ornithine decarboxylase medium (*8*), Christensen urea slants, MR-VP broth, Simmons citrate, triple sugar iron agar slants, gelatin, malonate broth, lysine decarboxylase and arginine dihydrolase semisolid media, and phenylalanine agar. Kovac reagent was used to detect the presence of indole in the tryptone tubes. Beta-galactosidase was detected from triple sugar iron slants (*11*). One percent

TABLE 1. Yersinia enterocolitica *strains tested.*

Antigen	Origin	Contributor
Unknown	Human (face)	ATCC 9610
Unknown	Human (blood)	ATCC 23715
0:8	Human (blood)	ATCC 27729
0:8	Stream water	ATCC 27739
0:8	Human, United States	E. J. Bottone
0:17	Human, United States	
0:17	Human, United States	
0:3[a]	Human, Canada	S. Toma
0:8	Human, Canada	
0:9	Unknown, Canada	
0:6,30	Human, Canada	
0:4,32	Human, Canada	
0:5,27	Human, Canada	
0:16	Stream water, U.S.	S. Harvey
0:3	Swine, Japan	M. Tsubokura
0:3	Swine, Japan	
0:5	Swine, Japan	
0:5	Swine, Japan	
0:17	Vacuum-packed meats United States	C. Vanderzant
0:17	Vacuum-packed meats United States	
Nontyped	Vacuum-packed meats United States	
0:20	Vacuum-packed meats United States	
Nontyped	Vacuum-packaged meats United States	
0:3[b]	Human, Europe	H. H. Mollaret
0:9[c]	Human, Europe	

[a]Phage type 9b. Different from European 0:3 serotype.
[b]Nilehn's biotype 4.
[c]Nilehn's biotype 2.

concentration of the following carbohydrates was sterilized in Difco phenol red broth base: glucose, mannitol, inositol, sorbitol, rhamnose, raffinose, salicin, meliboise, lactose, amygdalin, sucrose, trehalose, adonitol and dulcitol. Ten percent solutions of xylose and arabinose were filter sterilized and 0.5-ml volumes were pipetted into 4.5 ml of sterile broth base. The inoculated media were incubated at 25 and 37 C, and reactions were read after 2, 4 and 7 days. Differential charts and schemes presented in Ewing (*9*), Darland et al. (*7*), Bottone (*3*), and Brenner et al. (*5*), were used for identification of all strains.

Bacterial preparation for miniaturized systems

Each strain was inoculated into BHI and incubated at 25 C for 24 h. After incubation, a loopful of cells was aseptically streaked onto Difco Plate Count Agar and incubated at 25 C for 24 h.

API 20E system

The tests in the API 20E system were done according to manufacturer's instructions with some slight variations. Three to six colonies from Plate Count Agar were transferred into 10 ml of sterile distilled water and vortexed until the cells were fully dispersed. Using a sterile Pasteur pipette, the suspension was inoculated onto an API 20E test strip containing the 20 standard biochemical tests. The test strip was incubated at 37 C for 18-24 h. After incubation, appropriate reagents were added and the reactions recorded. All strains were identified to the species level by using the API Profile Recognition System.

Minitek system

In this study, the 12 disks chosen were the basic set recommended by the manufacturer for the differentiation of *Enterobacteriaceae*. These disks included dextrose/nitrate, hydrogen sulfide/indole, ornithine, urea, lysine, arabinose, rhamnose, inositol, phenylalanine, citrate, *O*-nitrophenyl-β-D-galactopyranoside (ONPG), and malonate. These substrates in the Minitek system were used following manufacturer's suggestions with a slight modification. Two Minitek inoculum broths (1.0 ml per vial) were aseptically transferred into a third vial. From each plate count medium, three to six colonies were dispersed into the inoculum broth (3.0 ml) and vortexed. With the aid of a Miniek pipette gun, 0.05-ml volumes were dispensed into each well. The plates were incubated at 37 C for 18-24 h. The addition of reagents and interpretation of the results for the 14 tests were followed according to manufacturer's recommendations. The BBL Minicoder was used for identification of each strain.

RESULTS AND DISCUSSION

Twenty-five typical and atypical strains of *Y. enterocolitica* isolated from human and nonhuman sources were obtained from various geographical locations throughout the world (Table 1). Nine atypical strains were used in this study which utilized rhamnose, raffinose, melibiose or citrate. Typical strains included the most predominant serotypes isolated in the United States [0:8 (*3*)], Europe [0:3 and 0:9 (*25*)], Canada [0:3, phage type 9b (*30*)], and Japan [0:3 (32)]. Before this study, biochemical results from conventional methods identified all the strains as *Y. enterocolitica* (data not presented).

By using the Minicoder and following the manufacturer's recommended identification procedure, the results from the 12 Minitek disks correctly identified 80% (20/25) of the strains. As indicated in Table 2, four of the five strains misidentified by Minitek were atypical strains isolated from vacuum-packaged meats (*13*). In contrast to the atypical strains isolated by Bottone et al. (*4*) and Harvey et al. (*15*) which produce acid from rhamnose at 25 C, the vacuum-packaged meat isolates utilize rhamnose at 25 and 37 C (*13*). Therefore, the Minitek sysem identified these four strains as *Citrobacter freundii* due to their rhamnose- and inositol-positive reactions at 37 C. By using the 12 recommended Minitek disks for the identification of *Enterbacteriaceae*, Chester and Evans (Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C168, p. 305) correctly identified all 33 *Y. enterocolitica* strains representing four DNA relatedness groups based on reactions for rhamnose, raffinose, medibiose, sucrose and citrate. However, the Minicoder system was not flexible enough to account for the atypical rhamnose-utilizing isolates from the vacuum-packaged meats, and consequently incorrectly identified them in this study.

TABLE 2. Y. enterocolitica *strains not identified by Minitek*[a].

Antigen type[c]	Origin	Incorrect identification	Incorrect reactions on Minitek[b]
0:5	Swine, Japan	*Escherichia coli*	Urease −
0:17	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
0:17	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
0:20	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
Nontyped	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+

[a]All strains were correctly identified by the API 20E system.
[b]Abbreviations: rham: rhamnose; ino.: inositol.
[c]The strain isolated from swine in Japan was a typical *Y. enterocolitica* strain whereas the isolates from vacuum-packaged meats represented atypical strains.

The other strain not identified as *Y. enterocolitica* by Minitek was the 0:5 serotype isolated from swine in Japan. The Minitek system identified this typical strain as *Escherichia coli* (Table 2). This disagreement was caused by a false-negative urease reaction which has been shown to occur with other *Enterobacteriaceae* members for Minitek (*27*).

The API 20E correctly identified all the *Y. enterocolitica* strains (Table 2). The atypical isolates from vacuum-packaged meats failed to use rhamnose on the API 20E strips at 37 C (unpublished data). Consequently these atypical strains displayed a biochemical reaction scheme resembling a typical *Y. enterocolitica* incubated at 37 C. Therefore, the API Profile Recognition System correctly identified all the atypical and typical strains of *Y. enterocolitica*.

Y. enterocolitica is a human pathogen that can grow anaerobically at refrigerated temperatures (4 C) in foods (*22,24*). An evaluation of *Y. enterocolitica* as a microorganism displaying public health significance in foods cannot be ascertained until more information is accumulated pertaining to its incidence in foods. Recently a rapid increase of atypical isolates has occurred in clinical (*4*), environmental (*15,19,21*) and food samples (*13,26*). Both typical and atypical strains have been recovered from stool cultures of patients who had acute abdominal disease (*3*). Therefore, a rapid and accurate method for identifying the increasing atypical as well as typical strains could prove valuable to the clinical and food microbiologist. The Minitek and API 20E are designed to be convenient and rapid diagnostic systems. The ability of either of these systems to accurately identify *Y. enterocolitica* strains could be very advantageous. In this study, the 12 basic Minitek disks representing 14 tests identified 93.8% of the typical *Y. enterocolitica* strains. When identifying the nine atypical strains, the Minitek system correctly differentiated only 55% of these strains. However, by supplementing the 12 basic Minitek disks with two conventional tubed tests consisting of Voges-Proskauer and motility incubated at 25 and 37 C, possibly these atypical isolates which were misidentified as *C. freundii* could be correctly identified as *Y. enterocolitica*. The API 20E system accurately identified 100% of the typical and atypical strains of *Y. enterocolitica* used in this study. The data presented in this paper indicate that Minitek and API 20E systems could prove beneficial for identifying *Y. enterocolitica* strains.

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FOODSERVICE COMMITTEE

The Foodservice Committee was formed in December 1977, at the request of the *Journal* Editor and charged with the following responsibilities:

1. To identify timely foodservice topics that should be discussed in the *Journal of Food Protection*.
2. To identify authors with the needed expertise to prepare papers on the various topics.
3. To assist with the solicitation of manuscripts and to encourage people doing research in foodservice to submit appropriate research papers to the *Journal*.

Membership on the committee consists of: C. Dee Clingman, Chairman, Director, Food Protection Programs, National Institute for the Foodservice Industry; K. J. Baker, R. S., Senior Food Consultant, Food and Drug Administration; Ruth S. Dickie, R.D., Department of Continuing Medical Edueation, University of Wisconsin; Dorothy Ellis, R.P.Dt., Food Technology Division, George Brown College; Dave Hartley, Director, Public Health, National Automatic Merchandising Association; Earl Helmreich, Food Protection Unit, Ohio Department of Health; Fred Mitchell, Chief, Hotels, Resorts & Restaurants, Minnesota Department of Health; Roy Moser, Extension Food Technologist, University of Hawaii; Robert Pickenpack, Director, Product Safety, General Mills, Inc.; Barry Preswick, Quality Assurance Supervisor, McDonald's Corporation; Thomas Schafer, Director, Quality Control & Sanitation, Pizza Hut, Inc.; Oscar Snyder, Associate Prof., Department of Food Science and Nutrition, University of Minnesota; Gail Terreri, Microbioloist, Arthur Treacher's Fish and Chips; Nan Unklesbay, Assistant Prof., Department of Food Science and Nutrition, University of Missouri; James C. White, Professor, Hotel Administration, Cornell University.

The first official activity of the committee was the completion of an Idea Explosion Form by each committee member. This activity was accomplished through written correspondence. Over one hundred ideas and comments were received by the Chairman as the result of this inquiry.

On August 14, 1978, the committee held its first meeting in Kansas City, Missouri in conjunction with the IAMFES annual meeting. The minutes of the meeting are contained below. These minutes contain specific committee recommendations and are the initial activities of the committee.

It is anticipated that future committee activities will be completed largely through written correspondence. However, a committee meeting is planned for early 1979, and again in August 1979, in Orlando.

COMMITTEE MINUTES

The committee was called to order at 2:00 PM on Monday, August 14, 1978, in Kansas City. Those in attendance were K. J. Baker, Dee Clingman, Pat Franks, Robert Pickenpack, Gail Terreri, and Nan Unklesbay.

1. It was requested that the Chairman obtain a breakdown, if possible, of IAMFES membership as far as the major disciplines are concerned (regulatory, industry, education, student, etc.). The Chairman indicated that he would attempt to obtain this information through the IAMFES Executive Secretary.
2. It was generally felt that an index or catalog of foodservice articles which appeared in the *Journal* be published. Such an index would aid in the location of articles and serve as a reference to the committee on future article solicitation. Mr. K. J. Baker volunteered to research past issues of the *Journal* from five years ago to present and compile an indexed, cross referenced catalog of foodservice articles by general topic. A tentative completion for this project is November 1, 1978.
3. It was proposed that the committee be considered as a publication review committee for all articles submitted to the *Journal* on foodservice.
4. It was agreed that all committee members should request relevant articles from speeches which are given at various organizational functions and relate to foodservice food protection.
5. It was recommended that the area of continuing education be reviewed as its application within the general structure of the *Journal*. Items such as quizzes, essay type examinations, or additional technical information should be examined.
6. It was recommended that a student award and an individual membership award be established and presented each year for the best overall article on foodservice food protection. Annual winners would be determined by the committee. The awards would be presented at the IAMFES Annual Meeting during the awards banquet or in the foodservice section. Further discussion on this matter will be taken up with the *Journal* Editor and the IAMFES Executive Board.
7. The following areas were discussed with reference to future *Journal* articles on foodservice food protection:
   A. Within one general topic area there could be a number of articles written in this topic area by different authors at different technical levels.
   B. There appears to be a need to encourage more management and marketing level people, who control purse strings within corporate structures, to share their concerns, fears, and doubts regarding foodservice food protection.
   C. The areas of product liability and the legal aspects of consumer food protection should be addressed from a legal standpoint.
   D. Summaries of the Food and Drug Administration Compliance Reports should be submitted to the *Journal* for publication.
   E. Articles on the quality assurance hierarchy within various companies as well as the philosophy of these organizations should be described for the readership.
   F. Articles should be solicited and encouraged based on the Center for Disease Control's listing of the most common factors involved in foodborne illness.
   G. It was generally felt that the incorporation of more photographs would enhance the visual appearance of articles.
8. The following recommendations apply to the *Journal* design and management:
   A. There was some concern regarding the design of the Table of Contents for the *Journal*. Some committee members felt that it was difficult to scan the *Journal* rapidly for relevant articles. It was suggested that the Table of Contents be organized more effectively so that the reader can rapidly find their area of interest. An idea was to insert adjacent

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