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Comparison of Miniaturized Multitest Systems with Conventional Methodology for Identification of *Enterobacteriaceae* from Foods

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Four miniaturized multiple test systems were compared with tube methodology used to identify *Enterobacteriaceae* encountered in foods. Identification aids supplied with each system were used to assign names to isolates at the species level. For the 129 strains tested, the Minitek system demonstrated a 96.9% agreement with reactions in tubed media. The Inolex, Analytab, and PathoTec test systems exhibited 94.3, 93.8, and 92.7% agreement, respectively. Analytab identified 96.1% of the isolates to the species level, whereas the Minitek, PathoTec, and Inolex systems were able to identify 78.3, 32.6, and 27.1%, respectively. The results indicate that the Analytab and Minitek systems are acceptable substitutes for the tube methodology routinely employed in identifying enterics from foods. Although the PathoTec system might be used to screen isolates for their identity, neither the presently available PathoTec nor the Inolex systems should be substituted for current methodology when definitive identification of foodborne organisms is required.

Foods, in addition to providing the body with nutrients, serve as vehicles for transmission of microorganisms. Plants and animals, along with their natural microbial flora, become further contaminated via soil, water, sewage, and air as well as by contact with other plants and animals (5). Additional contamination of foods occurs during handling and processing. Many of these contaminants, especially those of comminuted meat products such as turkey (6) and ground beef (8a), are members of the family *Enterobacteriaceae.*

Within the last 8 years, several miniaturized test systems for speciation of isolates within the *Enterobacteriaceae* have been made commercially available. The literature contains reports of several studies (1, 7, 8, 9, 10, 12-17) designed to compare these test systems with conventional tube methodology for the identification of clinically isolated organisms. However, there are few reports dealing with the routine use of such test systems in food microbiology laboratories.

This study was undertaken to compare four commercial enteric identification systems with conventional methodology for speciation of isolates from foods. (This paper was presented in part at the 77th Annual Meeting of the American Society for Microbiology at New Orleans, La., May 1977).

MATERIALS AND METHODS

Cultures. All organisms tested were lyophilized isolates from comminuted beef, pork, or turkey, with the exception of two *Edwardsiella* strains that were obtained from the stock culture collection of the Food Hygiene Division at Letterman Army Institute of Research.

One week before testing, cultures were rehydrated and examined for purity on blood and eosin methylene blue (BBL, Cockeysville, Md.) agars and then maintained on nutrient agar (Difco Laboratories, Detroit, Mich.) slants until they were tested.

Twenty-four hours before testing, cultures were inoculated into a 50-ml centrifuge tube containing 30 ml of Trypticase soy broth (BBL). After incubation at 37°C, cells were removed by centrifugation at 3,000 rpm for 30 min at 4°C. The supernatant was discarded, and the pellet was suspended in 20 ml of 0.85% saline. Inocula for the four test systems, as well as the tubed media, were prepared from this suspension.

Test systems. (i) API-20E (Analytab Products, Inc., Plainview, N.Y.). The API-20E system consists of a plastic strip holding 20 miniaturized tubes, each containing a lyophilized substrate for one of the following determinations: hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside; decarboxylation of lysine, ornithine, and arginine; utilization of citrate; production of hydrogen sulfide (H_2S); deamination of urea; deamination of tryptophan; production of indole; production of acetylmethylcarbinol; gelatin liquefaction; and the fermentation of glucose, mannitol, inositol, sorbitol,

rhamnose, sucrose, melibose, amygdalin, and arabinose. Before inoculation, each strip was placed in a plastic moisture chamber containing 5 ml of tap water. The inoculum was prepared by adding 0.2 ml of the organism, previously suspended in saline, to 5 ml of sterile distilled water. The inoculum was mixed and added to each well according to the manufacturer's instructions. Strips were incubated for 24 h at 37°C before any required reagents were added, and then the reactions were recorded. Isolates were identified to the species level by using the API Profile Recognition System.

(ii) Inolex Enteric 1 (Inolex Biomedical Division, Glenwood, Ill.). The Inolex Enteric 1 system consists of a plasticized card holding 10 capillaries containing dry substrates for the following biochemical tests: malonate utilization, glucose fermentation (RES), phenylalanine deamination, hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside, indole production, H_2S production, lysine and ornithine decarboxylation, deamination of urea, and fermentation of sucrose. Each card was placed in a plastic incubation chamber and inoculated with a cell suspension approximately 10^7 colony-forming units per ml as suggested by the manufacturer. After 24 h of incubation at 37°C, the necessary reagents were added to the capillaries, and all reactions were recorded. The Var-ident Binary Identification system was used for identification of strains tested with this product.

(iii) Minitek (BBL, Cockeysville, Md.). The Minitek system consists of a plastic plate containing 12 wells, a disk dispenser, humidors for incubation of plates, and inoculum broth. The system uses disks that have been impregnated with substrates to be tested. Although numerous substrate-containing disks are available, the 12 disks chosen for this study were the basic set recommended by the manufacturer, as follows: dextrose, *o*-nitrophenyl-β-D-galactopyranoside, H_2S, citrate, malonate, lysine, ornithine, arabinose, inositol, rhamnose, phenylalanine, and urea. Inocula were prepared by placing 2 drops of the saline suspension of cells into the inoculum broth. Plates were then inoculated and incubated according to manufacturer's instructions. After 24 h of incubation, reagents were added as required, and the reactions were recorded. The BBL Minicoder was used for identification of organisms tested.

(iv) PathoTec "Rapid I-D System" (General Diagnostics, Morris Plains, N.J.). The PathoTec Rapid I-D System consists of paper strips impregnated with substrates and indicator systems for detecting the presence of specific enzymes or metabolic end products, as follows: nitrite, phenylalanine deaminase, urease, H_2S, indole, lysine decarboxylase, acetylmethylcarbinol, malonate, cytochrome oxidase, and esculin hydrolysis. The cytochrome oxidase and esculin hydrolysis strips were not used in this study. For testing, 0.3 ml of a 1:10 dilution of the saline suspension prepared above was pipetted into each of eight test tubes (13 by 100 mm). The appropriate strips were added, and the tubes were incubated at 37°C for 4 h. After incubation, the strips were read as directed on the package insert, and the reactions were recorded. The PathoTec Rapid Identifier for *Enterobacteriaceae* was used to identify the isolates tested.

Conventional media. The following media were prepared according to the manufacturer's (BBL) instructions: triple-sugar iron agar, Simmons citrate agar, malonate broth, MR-VP broth, phenylalanine agar, and indole-nitrite medium with added agar for detection of motility. Moeller decarboxylase broth base (BBL) was used as a basal medium for the testing of lysine and ornithine decarboxylases and arginine dihydrolase. Urea broth base (BBL) was used for preparation of urea agar. A 1% tryptone broth (Difco) was used as the medium for indole production. Nutrient gelatin (Difco) was used for detection of gelatin liquefaction. The media for carbohydrate fermentation were prepared as described by Ewing and Davis (4). Inositol, sorbitol, rhamnose, amygdalin, melibiose, and arabinose were prepared in a final concentration of 0.5%, and glucose, sucrose, and mannitol were prepared in a final concentration of 1%.

All tubes were inoculated by pipette with 0.1 ml of the saline suspension of cells prepared above and were incubated at 37°C. Reactions were read at 24 h. Negative tubes were held at 37°C and re-examined daily for 10 days before being discarded.

Differential charts presented in Edwards and Ewing (2) and Ewing (3) were used for identification of the isolates tested.

RESULTS AND DISCUSSION

In this study, 129 strains of food isolates of *Enterobacteriaceae* (Table 1) were biochemically characterized, and reactions in each of the test systems were compared with those in conventional tubed media.

The API-20E system exhibited an overall agreement of 93.8% with standard tubed media. With the exceptions of melibiose and amygdalin, showing 85.8 and 75.4% agreement, respectively, the remaining 18 tests achieved a 91% or better agreement.

When individual reactions in the Inolex Enteric 1 card and standard methodology were compared, reactions for H_2S, indole, lysine decarboxylase, phenylalanine deaminase, *o*-nitrophenyl-β-D-galactopyranoside, and malonate indicated agreement of 96% or better. Reactions of urea, sucrose, and ornithine decarboxylase showed 87.3, 83.6, and 88.1% agreement, respectively. Only the RES capillary, used to determine glucose fermentation, demonstrated a 100% agreement with tubed media. With this system, indicator changes (especially for the decarboxylase reactions) were occasionally difficult to interpret. The overall agreement with standard media for the Inolex Enteric 1 System was 94.3%.

With the Minitek system, the only agreements below 96% were those of 88.8 and 84.3% for citrate and urea, respectively. The overall agreement between reactions in this system and those in tubed media was 96.9%.

H_2S and urea reactions were the only tests in

TABLE 1. *False reactions encountered with miniaturized systems*

Biochemical test	System[a]			
	API-20E	Inolex 1	Minitek	PathoTec
False positive reactions				
Citrate	0	-	1	-
Urea	1	2	0	8
H_2S	0	1	0	8
Indole	0	0	0	3
Lysine decarboxylase	1	2	0	0
Ornithine decarboxylase	1	12	0	-
Mannitol	2	-	-	-
Melibiose	9	-	-	-
Amygdalin	19	-	-	-
Arabinose	3	-	-	-
Voges-Proskauer	1	-	-	0
False negative reactions				
Citrate	5	-	1	-
Urea	3	0	2	5
Indole	2	3	0	1
Lysine decarboxylase	6	0	1	1
Ornithine decarboxylase	2	0	1	-
o-Nitrophenyl-β-D-galactopyranoside	4	2	1	-
Mannitol	1	-	-	-
Inositol	3	-	0	-
Sorbitol	3	-	-	-
Rhamnose	4	-	3	-
Saccharose	2	3	-	-
Melibiose	4	-	-	-
Amygdalin	3	-	-	-
Arabinose	1	-	1	-
Voges-Proskauer	9	-	-	0

[a] Number of false reactions for 129 tests. -, Substrate not contained in test system.

the PathoTec system that demonstrated agreement below 90%. Agreement between tubed urea slants and the reagent-impregnated strip was only 76.1%, and that for H_2S production was 88.8%. With the exception of a 90.3% agreement for the Voges-Proskauer reaction, the remaining strip tests demonstrated a 95.5% agreement or greater. The overall agreement between standard tube technique and this system was 92.7%.

False positive and false negative reactions obtained with each system can be seen in Table 1. The false positive and negative reactions observed with the API-20E system did not result in misidentification of the organisms and, therefore, must be compensated for within the analytical profile index. The false positive H_2S reactions obtained with the PathoTec strips did account for this system's misidentification of all *Proteus morganii* strains. All *Salmonella* strains tested were identified correctly by the API, Minitek, and PathoTec kits, while the Inolex system suggested additional testing to distinguish between *Salmonella* and *Edwardsiella* species. None of the false positive or negative urease reactions observed were with the *Salmonella* strains. These observations agree with a study of rapid identification of 110 foodborne *Salmonella* cultures (11).

Table 2 lists the number of correct identifications to the species level that was obtained with the use of the identification aid supplied with each product. Whereas the identification aids that complement the Minitek and API systems are designed to identify all isolates to the species level, those of the Inolex and PathoTec systems generally do not make distinctions within the *Klebsiella-Enterobacter-Serratia* group. The API Profile Recognition System was able to identify 96.1% of the enteric strains studied, whereas the Minitek, PathoTec, and Inolex aids could identify only 78.3, 32.6, and 27.1%, respectively, to the species level.

While the API-20E and Inolex Enteric 1 kits

TABLE 2. *Identification to species level of foodborne isolates using product identification aid*

Organism	No. of strains tested	Test system			
		API-20E	Inolex 1	Minitek	PathoTec
Escherichia coli	9	9	6	9	7
Citrobacter freundii	10	10	7	9	9
C. diversus	5	5	0	2	0
Salmonella enteritidis	10	10	0	10	10
Arizona hinshawii	9	7	1	3	1
Klebsiella pneumoniae	10	10	5	9	4
Enterobacter cloacae	11	11	3	8	1
E. aerogenes	4	3	0	0	0
E. hafniae	8	8	0	6	1
E. agglomerans	8	8	0	4	1
Serratia marcescens	10	10	0	10	0
Serratia liquefaciens	11	9	0	9	0
Proteus vulgaris	4	4	0	2	0
P. mirabilis	7	7	5	7	6
P. morganii	8	8	8	8	0
P. rettgeri	1	1	0	1	0
Providencia alcalifaciens	2	2	0	2	0
Edwardsiella tarda	2	2	0	2	2
Total identified		124	35	101	42

offer test batteries that have been preselected by the manufacturer, the PathoTec and Minitek systems allow the microbiologist an opportunity to select a specific series of tests from the substrates offered.

Based on these results, the Inolex Enteric 1 system should not be used to identify foodborne enteric organisms. The PathoTec system, which is readable in just 4 h, might be used to screen samples for the presence of certain genera, such as *Salmonella*. The API-20E and Minitek systems provide the food microbiologist accurate as well as time- and labor-saving systems for the definitive identification of enteric organisms present in food products.

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