JOURNAL OF CLINICAL MICROBIOLOGY, July 1980, p. 10-14
0095-1137/80/07-0010/05$02.00/0

Vol. 12, No. 1

Use of the API 20E System to Identify Veterinary *Enterobacteriaceae*

EVRYLL C. SWANSON AND MICHAEL T. COLLINS*

Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

A total of 503 veterinary enteric bacterial pathogens obtained from state veterinary diagnostic laboratories were tested on API 20E strips to determine whether this rapid microidentification system could be utilized for veterinary clinical microbiology. The API 20E strip accurately identified 96% of the veterinary isolates and misidentified 3%. Identifications by the API system and the diagnostic laboratories were in agreement in 83% of the isolates, disagreement on 16% of the isolates, and 1% were not identified by the API strip. Differences in identification occurred primarily in distinguishing between *Klebsiella* and *Enterobacter* and between *Enterobacter* and *Escherichia coli*. These disagreements were most often due to incorrect identifications by the diagnostic laboratory rather than by the API system. Biotype differences between human and veterinary isolates were compared. Significant differences were noted in several biochemical reactions. The main differences observed for *E. coli* isolates were in ornithine decarboxylase production and melibiose fermentation. The largest differences for *Salmonella* occurred in arginine dihydrolase production, citrate utilization, and inositol fermentation, whereas for *Klebsiella pneumoniae* the main differences were noted in urease production and nitrate reduction. These biotype differences, however, did not affect the accurate identification of organisms on the API strip.

A large portion of the work in a diagnostic laboratory consists of the identification of bacterial pathogens from the family *Enterobacteriaceae*. Veterinary diagnostic laboratory identifications generally rely on conventional tube media and lack the standardization provided by rapid microidentification systems. These microtest systems are designed for easier use and provide more rapid and accurate identifications (by use of a computer-generated data base). Rapid microtest systems could benefit a veterinary diagnostic laboratory, but these systems are designed specifically for human clinical isolates, and their applicability for identification of veterinary bacterial pathogens has not been established. Many diagnosticians believe that there are biotype differences between human and veterinary bacterial pathogens which would render rapid microtest systems less accurate for veterinary clinical microbiology. This study was undertaken to determine whether any significant biotype differences exist between veterinary enteric bacterial pathogens and human clinical isolates and whether the API system was capable of identifying these pathogens. The API 20E strip was chosen because of the large number of tests available (which was beneficial when comparing the biotypes between human and veterinary pathogens) and because of its proven accuracy (1, 6, 8, 11, 12).

MATERIALS AND METHODS

Cultures. Five-hundred three veterinary *Enterobacteriaceae* were collected from seven veterinary diagnostic laboratories across the country. Each culture was assigned a code number and lyophilized. The number and animal source of each organism tested are shown in Table 1.

Before testing on the API strip, lyophilized cultures were reconstituted with sterile Trypticase soy broth (BBL Microbiology Systems) and plated on blood agar. Cultures that were growing strongly were then tested without further passage. Cultures that grew weakly were passed once more on blood agar before testing.

API system. The API system (Analytab Products, Inc., Plainview, N. Y.) is composed of a plastic strip with 20 microtubes containing dehydrated biochemicals. It consists of the following tests: *o*-nitrophenyl-β-D-galactosidase (ONPG), arginine dihydrolase, lysine and ornithine decarboxylase, citrate, H_2S, urease, tryptophan deaminase, indole, Voges-Proskauer, gelatinase, and fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose. The strips were inoculated (with a 24-h culture from blood agar) and interpreted by the manufacturers recommended procedures. Each culture was also tested on API motility and O-F media and streaked onto a blood-MacConkey biplate. Positive reactions were converted to a seven-digit profile number, and identifications were made with the *API Profile Index* (fourth edition) and were compared with the diagnostic laboratories' identification. Code numbers not found in the API index were called into the

TABLE 1. *Organisms*[a] *and their sources used in this study*

Organism[a]	Total	Bovine	Porcine	Equine	Ovine	Caprine	Feline	Canine	Avian	Guinea Pig	Other	Source unknown
Arizona	3				1				1			1
Citrobacter freundii	13	5	3	2	1		1	1				
Citrobacter diversus-levinea	4	1		2								1
Enterobacter aerogenes	4			4								
Enterobacter agglomerans	10	3		5				1				1
Enterobacter cloacae	25	8	1	8		1	1	4	1		1[b]	
Escherichia coli	197	74	45	18	6	2	7	25	3	1		16
Edwardsiella tarda	2						2					
Hafnia alvei	2			1								1
Klebsiella oxytoca	8	1		2	1		1	1		1		1
Klebsiella ozaenae	1			1								
Klebsiella pneumoniae	45	6	4	15	1	1	1	14				3
Morganella morganii	1											1
Proteus mirabilis	23	4	3	1	1		1	11			1[c]	1
Proteus vulgaris	5	3		1	1							
Providencia rettgeri	1			1								
Providencia stuartii	3	2										1
Providencia alcalifaciens	2							2				
Salmonella sp.	117	17	7	14	1		1	9	12		12[d]	44
Salmonella cholerae-suis	28	16	4									8
Salmonella paratyphi B	2	1		1								
Salmonella pullorum	1											1
Serratia marcescens	2	2										
Serratia liquefaciens	1											
Yersinia enterocolitica	3								1			3

[a] As identified by the API 50E.
[b] Rabbit.
[c] Iguana.
[d] Monkey (nine), ferret (one), opossum (one), skunk (one).

API computer center for identification. When there was disagreement between API and the diagnostic laboratory, or when the profile number was not listed in the index, the culture was sent to the API reference center for further testing on a 50-test strip to obtain a final identification. When identifications by API and the diagnostic laboratory were in agreement, the identification was considered correct and not tested further.

Biotyping. A preliminary examination of the biotype differences between human and veterinary isolates was conducted. For each organism, the percentage of the isolates that were positive for each reaction was calculated. This percent positivity for the veterinary isolates was compared with the API percentage chart, which is based on reactions of clinical isolates of human origin (and is based solely on reactions obtained on the API strip). The significance of the difference in percentages for each reaction was determined by the chi-square test.

RESULTS

Comparison of API and diagnostic laboratory identifications. Bacterial identification agreements and disagreements between the API system and the diagnostic laboratories were reviewed. Results were separated on the basis of whether the identification was listed in the API Profile Index or whether it was necessary to consult the API computer center. This was done because an advantage of most rapid identification systems is the simplicity and efficiency of using an index. As shown in Table 2, we found that 78% of the isolates were identified the same by API and the diagnostic laboratory when using the index, and 83% were identified the same when computer identifications were included. Fifteen and sixteen per cent of the identifications differed when using the index and the total data base, respectively. Seven percent of the profile numbers were not in the API index, whereas only 1% were not in the computer data base. This 1% represented four organisms (three *Escherichia coli* and one *Enterobacter agglomerans*) which received an "unacceptable identification" designation by the computer, indicating that their biochemical test pattern did not resemble any taxa stored in the data base. Cultures with profile numbers not in the index but in the total data base consisted of unusual or possibly veterinary biotypes of *E. coli* (13 isolates), *Salmonella* (12 isolates), *E. agglomerans* (4 isolates), *Proteus* (3 isolates), *Klebsiella* (1 isolate), and *Providencia stuartii* (1 isolate). A common *E. coli* biotype that was not listed in the index was 5-144-113.

The API system and the diagnostic laboratories disagreed on 81 (16%) of the organisms. These conflicting identifications, along with the final identifications, are shown in Table 3. Fourteen of the different identifications varied only in species, and three of these were isolates identified as *Klebsiella oxytoca* by API, whereas the diagnostic laboratories identified them as *K. pneumoniae* (2 isolates) and *K. ozaenae* (1 isolate). *K. oxytoca* is a new species designation and is probably not in common usage in many laboratories. Disagreements in identification occurred most commonly when distinguishing between *Klebsiella-Enterobacter* identifications and *E. coli-Enterobacter* identifications. In most cases, there was no common cause for these differing identifications. Of the 81 conflicting identifications, the API system correctly identified 62 of the isolates, the diagnostic laboratories correctly identified 15, and 4 organisms were misidentified by both.

Considering all 503 veterinary isolates, the API 20E system correctly identified 96%, misidentified 3%, and was unable to identify 1%. The diagnostic laboratories correctly identified 87% and incorrectly identified 13% of the organisms.

Biotype differences. Biotype differences between human and veterinary isolates were compared in four taxa (*E. coli*, *Salmonella*, *K. pneumoniae*, and *Enterobacter cloacae*) where sufficient numbers of organisms were studied to permit statistical analysis. Table 4 lists the percent positivity for each biochemical reaction for each organism. These percentages were compared with the API percentage chart, which is based on human clinical isolates. Significant differences (at the 1% level) for *E. coli* isolates were noted in lysine decarboxylase (for which veterinary isolates were 91.2% positive and human isolates were 82.8% positive), ornithine decarboxylase (veterinary 60.3%; human 75.7%), sorbitol fermentation (veterinary 88.1%; human 94.4%), melibiose fermentation (veterinary 91.8%; human 67.1%), and arabinose fermenta-

TABLE 2. *Comparison of API 20E and diagnostic laboratory identifications*

Identifications	API index		Total API data base	
	No.	%	No.	%
Same	394	78	419	83
Different	76	15	81	16
Not listed	34	7	4	1

TABLE 3. *Identifications differing between API and the diagnostic laboratory*

API identification	Diagnostic laboratory identification	Final identification
18 *E. coli*	1 *Edwardsiella* 4 *K. pneumoniae* 5 *K. ozaenae* 4 *Enterobacter* 2 *E. aerogenes* 1 *Arizona* 1 *Y. enterocolitica*	18 *E. coli*
14 *K. pneumoniae*	3 *K. ozaenae* 6 *Enterobacter* 1 *E. liquefaciens* 2 *E. coli* 1 *E. agglomerans* 1 *C. freundii*	14 *K. pneumoniae*
3 *K. oxytoca*	2 *K. pneumoniae* 1 *K. ozaenae*	3 *K. oxytoca*
2 *K. ozaenae*	2 *K. pneumoniae*	2 *K. pneumoniae*
7 *Salmonella* sp.	4 *Citrobacter* 1 *E. aerogenes* 2 *Arizona*	7 *Salmonella* sp.
1 *Arizona*	1 *E. coli*	1 *E. coli*
1 *S. cholerae suis*	1 *Edwardsiella*	1 *Salmonella* sp.
5 *E. agglomerans*	3 *E. coli* 2 *K. pneumoniae*	1 *E. agglomerans* 2 *E. coli* 2 *K. pneumoniae*
4 *E. cloacae*	1 *E. aerogenes* 2 *K. pneumoniae* 1 *E. coli*	4 *E. cloacae*
3 *E. aerogenes*	2 *K. pneumoniae* 1 *E. cloacae*	3 *E. aerogenes*
7 *C. freundii*	3 *E. coli* 3 *Enterobacter* 1 *Salmonella* sp.	3 *C. freundii* 2 *C. freundii* 1 *E. coli* 1 *Salmonella* sp.
1 *C. diversus-levinia*	1 *Klebseilla*	1 *C. amalonaticus*
4 *P. mirabilis*	3 *P. vulgaris* 1 *C. freundii*	4 *P. mirabilis*
1 *P. rettgeri*	1 *C. freundii*	1 *P. rettgeri*
2 *P. vulgaris*	2 *P. mirabilis*	2 *P. vulgaris*
1 *P. stuartii*	1 *Proteus*	1 *P. rettgeri*
2 *P. alcalifaciens*	2 *P. mirabilis*	2 *P. vulgaris*
2 *S. dysenteriae*	1 *S. typhi-suis* 1 *E. agglomerans*	1 *Salmonella* group C 1 *E. agglomerans*
1 *H. alvei*	1 *E. coli*	1 *H. alvei*
1 Enteric group 8	1 *E. coli*	1 *E. coli*

TABLE 4. *Percentage of veterinary strains positive for each biochemical reaction*

Biochemical test	% Positive			
	E. coli (194)[a]	*Salmonella* sp. (113)	*K. pneumoniae* (41)	*E. cloacae* (20)
ONPG[b]	97.4	0.9	100	95
Arginine dihydrolase	2.6	91.2	0	100
Lysine decarboxylase	91.8	100	90.2	0
Ornithine decarboxylase	60.3	100	0	95
Citrate	0	15.0	92.7	95
H_2S	3.6	97.3	0	0
Urease	2.1	0	87.8	0
Tryptophan deaminase	0	0	0	0
Indole	97.4	0	0	0
Voges-Proskauer	0	0	97.6	100
Gelatinase	0	0	2.4	0
Glucose	99.5	100	100	100
Mannitol	99.5	100	100	100
Inositol	1.0	59.3	100	25
Sorbitol	88.1	99.1	100	95
Rhamnose	87.6	98.2	100	90
Sucrose	44.8	0	100	95
Melibiose	91.8	94.7	100	95
Amygdalin	4.6	0	100	100
Arabinose	99.5	99.1	100	100
Oxidase	0	0	0	0
Nitrate to nitrite	99.5	99.1	51.2	90
Nitrate to gas	0	0	46.3	5
Growth on MacConkey agar	100	100	100	100
Glucose, oxidative	100	100	100	100
Glucose, fermentative	100	100	100	100

[a] Numbers in parentheses are number of strains.
[b] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

tion (veterinary 99.5%; human 90.8%). Significant differences for *Salmonella* were observed in arginine dihydrolase (veterinary 91.2%; human 69.2%), citrate (veterinary 15.0%; human 75.4%), H_2S (veterinary 97.3%; human 85.7%), inositol fermentation (veterinary 59.3%; human 33.7%), and melibiose (veterinary 94.7%; human 78.2%). Reaction differences for *K. pneumoniae* biotypes were recorded for urease production (veterinary 87.8%; human 63.6%), indole production (veterinary 0%; human 19.2%), nitrate reduction to nitrites (veterinary 51.2%; human 100%), and nitrate reduction to nitrogen gas (veterinary 46.3%; human 0%). No significant differences were noted for *E. cloacae*.

When comparing veterinary *K. pneumoniae* biotypes with the human clinical biotypes obtained by DeSilva and Rubin (4), we found that the most common *K. pneumoniae* biotype for human and veterinary isolates was the same (5-215-773). For veterinary isolates, this biotype occurred at a frequency of 62%, whereas for human isolates it was observed in 51% of the isolates (4). This difference was significant at the 5% level.

The most common *E. coli* biotype frequencies are shown in Table 5. These differ somewhat from the biotype frequencies obtained by Davies (3) in his study of 574 *E. coli* strains, but the same patterns are present.

DISCUSSION

The differing identifications reached by the API system and the diagnostic laboratories were the result of misidentifications made either by the API system or by the diagnostic laboratory or by both. Of the 15 organisms that API misidentified, 6 isolates were given a "good likelihood, low selectivity" rating by the computer, meaning that several organisms could fit that profile number. In three of these cases, the correct identification was given as the second or third choice in the index, and the additional tests listed in the index probably would have given the correct identification. Also, when using this system, other factors such as colonial morphology and serology should be considered before reaching a final identification. Our identifications were based solely on reactions observed on the API strip.

The diagnostic laboratories misidentified 64 isolates. For economic reasons, most veterinary diagnostic laboratories utilize a minimal number of biochemical tests to identify each organism. Many isolates, particularly *E. coli*, are often identified solely by colonial morphology. In this instance, cultures resembling *E. coli* morphologically would be misidentified. In our study, 13% of the disagreements were organisms incorrectly identified as *E. coli* by the diagnostic laboratory. For other identifications, often only triple sugar iron agar slants and motility indole ornithine deeps are utilized. Occasionally, additional tests such as citrate, urease, *o*-nitrophenyl-β-D-galactopyranoside, or malonate were run, but use of these tests was highly variable between diagnostic laboratories. Identifications often are only made to genus and not to species.

In some cases, it was found that the diagnostic laboratory was misreading or misinterpreting its tests. For example, seven *K. pneumoniae* were identified as *Enterobacter* by one diagnostic lab-

TABLE 5. *Most common biotypes among 194 strains of E. coli*

Biotype	No. of strains	%
5 144 572	54	27.8
5 044 552	27	13.9
5 144 552	25	12.9
5 044 572	13	6.7
1 044 552	9	4.6
5 044 542	6	3.1
5 144 113	5	2.6
42 other biotypes	55	28.4

oratory. Their biochemical results indicated that the organism was motile and ornithine decarboxylase negative. The negative ornithine reaction is correct for *Klebsiella*, not *Enterobacter*, and retesting revealed that the organism was nonmotile instead of motile, thus also favoring a *Klebsiella* identification.

A few conflicting identifications probably also were due to the wrong culture being tested. Some cultures obtained were on primary isolation plates, and different colonies may have been tested. Also, some cultures appeared to have been mislabeled.

Overall, the API identifications were more accurate than the diagnostic laboratories' identifications. The API system correctly identified 96% of the veterinary isolates as compared with 87% correctly identified by the diagnostic laboratories. This 96% compares favorably to figures previously reported for the API system in identifying human clinical isolates (1, 5, 6, 10, 11, 12). Also, the API system gave more complete identifications (down to species) and was more consistent due to the *Profile Index*.

The applicability of the API strip for biotyping has received conflicting opinions (2–4, 9, 10). It has been criticized because of its lack of reproducibility when testing the same organism several times. We compared the biotypes of a population of organisms rather than individual biotypes, thereby reducing the variability within each biochemical reaction. Also, the percentage obtained for each biochemical reaction was generated in the same manner as for the human isolate percentages that are given in the API comparison chart. Although significant reaction differences were observed (as noted in the results), they did not affect the correct identification of the organisms by the API index.

Since the API system can accommodate many different biotypes, it appears to be capable of identifying veterinary bacteria in the family *Enterobacteriaceae* with a high degree of accuracy. This system is able to identify pathogens with equal or better accuracy as compared to conventional methods used by most diagnostic laboratories. It is also advantageous in that it is a very standardized system. The strip is always inoculated in the same manner, it contains a standard set of 20 biochemical tests, and it is interpreted with the API Index. In this respect, results obtained within one laboratory and between different laboratories with API would be standardized and easily comparable. Although the API 20E strip is cheaper to use than running a comparable set of biochemical tests (7), there may be a slight increase in cost for diagnostic laboratories that use only a minimal battery of tests. This cost would be offset by the convenience of the API strip, its accuracy, and the standardization of results obtained. It is concluded that a rapid microtest system such as API 20E is feasible for identifying veterinary bacterial pathogens from the family *Enterobacteriaceae*.

ACKNOWLEDGMENTS

This research was supported in part by Public Health Service Biomedical Research Support Grant 5-507-RR-05458-15 from the National Institutes of Health and Analytab Products.

We thank the following laboratories for providing us with veterinary enteric organisms: Animal Disease Laboratory, Centraliae, Ill.; Wyoming State Veterinary Laboratory; and the Veterinary Diagnostic Laboratories at University of Minnesota, University of Georgia, Kansas State University, Iowa State University, and especially Colorado State University.

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