Evaluation of Two Rapid Methods for Identification of Commonly Encountered Nonfermenting or Oxidase-Positive, Gram-Negative Rods

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Received for publication 24 August 1977

The ability of the expanded API-20E and the Oxi-Ferm System to identify 176 isolates of nonfermenting or oxidase-positive, gram-negative rods from 17 species or groups was studied. The expanded API-20E, without referral to a computer reference center, was able to identify 61.4% of the isolates to the species level. If reference to the computer center was utilized, an additional 25% could be identified. Of the isolates examined, 13.8% were misidentified, partially identified, or assigned no identification. Those assigned to the incorrect genus constituted 0.5% of the isolates tested; those assigned to the correct genus, but the wrong species, constituted 0.5%; 4.5% were placed in the correct genus with no species given; and 7.9% were assigned no identification. The Oxi-Ferm System was able to assign 75% of the isolates to the correct species without further testing, and an additional 19.3% required additional testing for correct identification. Those assigned to the incorrect genus represented 3.4% of the isolates tested; 11.1% were assigned to the correct genus, but the wrong species; and 1.1% were assigned to the correct genus, with no species indicated.

Over the last decade the nonfermenting or oxidase-positive, gram-negative rods have emerged as significant problems for clinical microbiologists. Because of personnel or other resources, many clinical facilities were ill equipped to confront the problems associated with the identification of organisms in this group. The traditional methodology for the complete identification of these organisms was cumbersome and required a large variety of media, which were often not routinely available to smaller hospitals. Therefore, identification was often neglected entirely when an antimicrobial susceptibility test was available. Although this procedure may have been adequate for patient recovery, it yielded no data to the hospital epidemiologist responsible for controlling nosocomial infections. As the role of these organisms became better defined and interest increased, a number of computerized rapid identification systems were developed and marketed.


MATERIALS AND METHODS

A total of 176 isolates of commonly encountered nonfermenting or oxidase-positive, gram-negative rods were selected from clinical isolates submitted to the Division of Diagnostic Microbiology, South Carolina Department of Health and Environmental Control, for identification. Each isolate was carefully characterized by using established criteria and techniques (1-4, 6, 7). The Oxi-Ferm System and the API-20E expanded by the addition of MacConkey agar, motility medium, and media for the detection of glucose oxidation and fermentation have been described (5; Dowda, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, p. C87, p. 50). The products were used exactly according to the manufacturer's instructions, with the exception that organisms that were oxidase negative were inoculated directly into the Oxi-Ferm System without first being tested in the Enterotube System (Roche Diagnostics), which is the normal procedure in clinical laboratories that utilize the Enterotube and Oxi-Ferm
Systems for the identification of gram-negative rods. This was done because the organisms used in this study had already been carefully characterized by conventional techniques. In clinical laboratories, especially in those in which microbiologists have difficulty recognizing oxidase-negative, nonfermenting, gram-negative rods based on their morphology and growth characteristics, the manufacturer's directions should be followed explicitly. Inocula for the systems were obtained from 24-h growth on plates of either heart infusion agar, 5% rabbit blood agar, or chocolate agar. Single, well-isolated colonies were generally used, but in some instances more than one colony was needed to obtain a sufficient inoculum for the Oxi-Ferm System. In this case, identical, well-isolated colonies were used. After inoculation, each system was incubated at 35°C in a room-air incubator. In the case of the expanded API-20E, isolates that gave four or more positive reactions after 24 h of incubation were encoded, whereas those isolates that gave less than four positive reactions were incubated for a total of 48 h, at which time they were encoded and identified, from the appropriate sections of the Analytical Profile Index (revised May 1976). In the case of the Oxi-Ferm System, inoculated tubes containing oxidase-positive organisms were incubated for 48 h, after which time the reactions were read and encoded and identifications were determined by using The Computer Coding and Identification System for Oxi-Ferm Tube (second edition). Oxidase-negative strains were examined after 24 h of incubation and again when the tubes had been incubated for a total of 48 h.

Code numbers not found in the API Analytical Profile Index were submitted via telephone to the API Computer Reference Center for identification. Additional biochemical tests or antimicrobial susceptibility tests needed by the Oxi-Ferm System were performed according to the manufacturer's instructions.

A strain was considered correctly identified when it was identified to the species level, except in the case of Moraxella, in which identification was considered adequate when the genus was identified, since neither system claims species-level identification for most members of this genus.

RESULTS

The ability of the expanded API-20E and the Oxi-Ferm System to identify commonly encountered nonfermenting or oxidase-positive, gram-negative rods is summarized in Table 1. The API-20E System correctly identified 40 of the 44 strains of glucose-oxidizing Pseudomonas aeruginosa tested. Sixteen of the correctly identified strains were identified after 24 h of incubation. The strains incorrectly identified were assigned to the Pseudomonas fluorescens group, which contained P. fluorescens, P. putida, and a few strains of P. aeruginosa, on the basis of the incomplete reduction of nitrate to gas. The single isolate of P. aeruginosa incorrectly identified by the Oxi-Ferm System was due to delayed glucose and xylose oxidation and was identified as Achromobacter sp. Both systems were able to identify five of the six strains of brown-pigmented, non-glucose-oxidizing P. aeruginosa. The Oxi-Ferm System failed to correctly identify an isolate that was arginine dehydrolase negative in the system, calling it Achromobacter xylosoxidans. The API-20E failed to identify an isolate due to the lack of either a positive urea or a positive gelatin. The isolate was, however, identified as a Pseudomonas sp. Three of the five strains of glucose non-oxidizing P. aeruginosa were identified by the expanded API-20E System with computer assistance after 24 h of incubation. All 14 strains of P. fluorescens were identified correctly by the Oxi-Ferm System. One strain was misidentified by the API-20E and was a urease-positive isolate that was identified as P. aeruginosa. A single isolate of P. fluorescens could be identified after 24 h of incubation. Both systems correctly identified the 23 isolates of Pseudomonas maltophilia included in this study. After 24 h of incubation, the Oxi-Ferm System could identify correctly 14 of the 23 isolates, the remainder requiring an additional 24 h of incubation. The expanded API-20E required 48 h of incubation for the identification of all isolates. Neither system was able to satisfactorily identify Pseudomonas cepacia. The API-20E was able to correctly identify to the species level three of the six isolates included. The other three isolates were identified as Pseudomonas sp. The Oxi-Ferm System correctly identified four of the six isolates, the balance being identified as Pseudomonas sp.

The Oxi-Ferm System correctly identified all 32 strains of Acinetobacter included in the study. A total of 18 of the 25 isolates of Acinetobacter calcoaceticus var. anitratum and 4 of the 7 isolates of A. calcovaceticus var. lwoffii could be identified after an incubation period of 24 h. The API-20E failed to identify two strains of A. calcoaceticus var. anitratum. Both of these strains gave positive ortho-nitrophenyl-β-D-galactosidase and urease reactions and were assigned no identifications. The API-20E correctly identified all nine strains of A. xylosoxidans. The Oxi-Ferm System was unable to perform adequately with this group of isolates, identifying only five. Two isolates failed to oxidize xylose in 48 h and were identified as Achromobacter sp. biotype 1, and another failed to produce gas or oxidize xylose within 48 h and was identified as Alcaligenes faecalis. One strain gave a positive arginine dehydrolase reaction in the Oxi-Ferm System and was identified as a Pseudomonas sp. Both systems identified the four strains of A. faecalis.
and two strains of Bordetella bronchiseptica included in the challenge group.

Members of the genus Moraxella were uniformly identified by the Oxi-Ferm System to the genus level. However, only 5 of the 11 strains tested were correctly identified by the API-20E. This was due to a variety of unexplained positive reactions, most notably arginine dehydrolase and assorted sugar fermentations. Cultures were purified and retested with equally confused results. Re-identification with conventional techniques confirmed the identification of the isolates as members of the genus Moraxella.

Three isolates of group Ve-1 were included in the study. The Oxi-Ferm System identified all three after 48 h of incubation, but none could be identified after 24 h of incubation; the API-20E was unable to identify any of these strains. All three were negative for nitrate reduction in the API-20E System, and one gave a positive gelatin reaction. Although these are not uncommon variations, the system could not identify isolates of Ve-1 giving these reactions. No other identification was assigned. The Oxi-Ferm System identified all three isolates of group III. The expanded API-20E identified two of these three strains, and the third, giving a positive ortho-nitrophenyl-β-D-galactosidase reaction, could not be identified.

Aeromonas hydrophila and Pleisomonas shigelloides were correctly identified by both systems. Vibrio parahaemolyticus was identified by the Oxi-Ferm System, but the API-20E failed to identify either of the two strains included. Only one strain would ferment glucose, and both were negative for citrate utilization in the API-20E. One strain that reduced nitrate to nitrite was identified as A. hydrophila, and the other was not assigned an identification. Flavobacterium sp. (group IIB) was represented by four isolates, all of which were identified by both systems. There was some difficulty in identifying these with the expanded API-20E in that all four isolates gave a false positive reaction for nitrate reduction, probably due to the production of a primary amine. Two of four strains of Pasteurella multocida were not identified by the Oxi-Ferm System. These were misidentified due to the lack of detectable indole production. The isolates were assigned an identification of Pasteurella ureae. The API-20E failed to identify one isolate because a positive arginine dehydrolase reaction was obtained, and no identification could be assigned.

The expanded API-20E without reference to the computer service was able to identify to the species level 108 of the 176 isolates tested (61.4%). Of the remaining strains, 44 (25.0%)
could be identified to the species level if the reference center was used. Twenty-four (13.6%) could not be correctly identified to the species level with this system. Twenty-three isolates (13.0%) could be identified with or without computer assistance after an incubation period of only 24 h, the balance requiring the full 48-h incubation period.

The Oxi-Ferm System was able to identify to the species level 132 (75.0%) of the strains examined without additional testing. An additional 34 strains (19.3%) could be identified by additional testing, for a total correct identification of 166 strains (94.3%). Of the 58 strains of oxidase-negative bacteria included in this study, the Oxi-Ferm System was able to identify 36 (62.0%) after an incubation period of 24 h. Strains requiring additional information, which did not require the inoculation of additional media, but rather only observations of existing parameters (e.g., runny growth, pigment, colony morphology), were included with the group needing no additional testing.

**DISCUSSION**

The Oxi-Ferm System had no single reaction that repeatedly caused major numbers of misidentifications due to inappropriate reactions. The indole reaction, which utilizes Kovac reagent, gave weak positives with *Flavobacterium* sp. and *P. multocida*. This problem resulted in the misidentification of two strains of *Pasteurella*. Delayed xylose or glucose oxidation was responsible for the misidentification of one strain of *P. aeruginosa* and two strains of *A. xylosoxidans*, which were all identified as *Achromobacter* sp.

In the case of the expanded API-20E, problems were encountered with false positive arginine dehydrogenases in two species, resulting in the lack of identification for seven strains. Positive *ortho*-nitrophenyl-β-D-galactosidase reactions were responsible for the lack of identification of three isolates. Two strains of *V. parahaemolyticus* either were not identified or were misidentified due to a lack of evidence of citrate utilization. Seven errors in identification were related to the reduction of nitrate, but most of these were not due to false positive or false negative reactions, but rather to the lack of adequate alternatives in the computer data base of the system.

Unfortunately, in many laboratories, identifications obtained from rapid systems are taken as absolute. This is especially true when microbiologists lack knowledge about the basic differential characteristics of so diverse a group as the nonfermenting or oxidase-positive, gram-negative rods. Therefore, the problem of misidentification versus no identification or incomplete identification becomes important. Table 2 summarizes the incomplete or incorrect identifications obtained in this study.

The API-20E failed to identify 24 strains. Of these, 8 were identified as belonging to the correct genus or group within the genus, but no species was determined. One strain was specifically identified as belonging to an improper species in the correct genus; a single isolate was placed in the incorrect genus, and fourteen strains were assigned no identification. Therefore, of the 176 isolates examined, only 0.6% were identified as being members of the incorrect genus; 4.5% were identified to the correct genus or group, with no species assigned; 0.6% were assigned to the wrong species, but correct genus; and 7.9% were assigned no identification whatsoever.

The Oxi-Ferm System failed to identify 10 of the isolates tested. Of these, six were placed in the wrong genus; two were assigned to the correct genus, but the wrong species; and two were assigned to the proper genus, with no species given. Therefore, of the 176 isolates examined, 3.4% were identified as being members of the incorrect genus; 11.1% were placed in the correct genus, but wrong species; and 11.1% were assigned to the correct genus, without any species designation. Although these data do not indicate that a serious problem exists with misidentification, they do strongly suggest that care must be taken to assure that the organisms being examined fit the general criteria for organisms identified by the data base of the system, with the understanding that a certain number of organisms in this study were, in fact, incorrectly identified.

In this study, the expanded API-20E was able to identify to the species level 86.4% of the isolates studied. The Oxi-Ferm System was able to identify to the species level 94.4% of this

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<th>Table 2. Incorrect or incomplete identifications by expanded API-20E or Oxi-Ferm System</th>
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* Numbers in parentheses indicate the percentage of 176 isolates included in this study.
challenge group. Therefore, it would appear that the two systems represent valuable tools for clinical microbiologists, especially for those microbiologists without adequate resources or experience to use the more definitive conventional methodologies.

LITERATURE CITED


