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Evaluation of an Automated, Computerized System (AutoMicrobic System) for *Enterobacteriaceae* Identification

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The automated and computerized AutoMicrobic system (AMS; Vitek Systems, Inc., subsidiary of McDonnell Douglas, Hazelwood, Mo.) was evaluated as a means of identifying the *Enterobacteriaceae*. The Micro-ID system (General Diagnostics, Morris Plains, N.J.) and, when necessary, conventional tubed media were used for comparison. Identification by AMS and Micro-ID differed in only 12 of 1,528 isolates (0.8%). Disagreements occurred primarily with *Enterobacter* spp. Precision testing of the AMS showed only 1 of 72 tests (1.4%) deviating from the expected. The AMS was found to be an accurate and precise method for the identification of *Enterobacteriaceae*.

The AutoMicrobic system (AMS; Vitek Systems, Inc., subsidiary of McDonnel Douglas, Hazelwood, Mo.) is an automated, computerized system for the detection, enumeration, and identification of microorganisms. The AMS is attractive because it is fully automated and allows the processing of a large number of samples with little manual work. The instrument makes use of a card containing an array of media sealed in small transparent wells. Solid state optics monitor color and density changes in each well during incubation, and a minicomputer then processes the data. Initial evaluation of the AMS was undertaken with clinical and simulated urine specimens for enumeration and identification (1, 8, 9). An expansion of the capability of the system included the introduction of a biochemical card for the identification of the *Enterobacteriaceae*. In a collaborative study by Isenberg et al. (7), the system was recently evaluated in six separate laboratories by using 170 stock cultures. The cultures were identified by the *Enterobacteriaceae* Biochemical Card (EBC) and either the standard method of Ewing and Martin (6) or the API system (Analytab Products, Plainview, N.Y.). Each of the laboratories also identified all clinical isolates belonging to the *Enterobacteriaceae* by the EBC during the study period and compared these with the method in use in their laboratory at the time. The accuracy of the EBC was 97.8% with the stock cultures and 96.4% with clinical isolates as compared with the different methods used in the various laboratories. A detailed analysis of the clinical isolates was not possible in that paper since the number of variables was so great. During the time of the investigation we were also comparing the EBC with the Micro-ID method (General Diagnostics, Morris Plains, N.J.) which was currently in use in our laboratory. The Micro-ID has been described and favorably compared with the API 20E system and conventional media and is a frequently used approach to the identification of the *Enterobacteriaceae* (2-5). An important advantage of the two methods is that they both identify the *Enterobacteriaceae* in short periods of time, 4 h for the Micro-ID and 8 h for the EBC. The ability of the AMS-EBC system to repeatedly identify the same organism was also tested with selected *Enterobacteriaceae*.

MATERIALS AND METHODS

Microorganisms. We tested 1,528 isolates of *Enterobacteriaceae* comprising 24 species. During the period of study, 1,487 strains were isolated from clinical specimens submitted to the clinical microbiology laboratory, and 41 consisted of stock cultures. Organisms used for repeatability testing were *Escherichia coli*, *Proteus mirabilis*, *Providencia stuartii*, *Salmonella enteriditis*, *Serratia liquefaciens*, and *Klebsiella pneumoniae*. Stock cultures, maintained in defibrinated rabbit blood at −70°C or lyophilized, were subcultured three times on blood agar plates before testing. Saline suspensions of microorganisms adjusted to no. 1 and no. 0.5 McFarland standards were used to inoculate the AMS-EBC and Micro ID systems, respectively.

AMS. The instrumentation and functions of the AMS have been described in detail (1). The AMS is an integrated modular system with a diluent dispenser, filling module, reader-incubator, computer control module, and data terminal. The disposable kit consists of a plastic card containing lyophilized media in small growth chambers and a sample injector. The EBC, which identified microorganisms of the family *Enter-*

obacteriaceae, has been described in detail by Isenberg et al. (7). Most of the biochemicals in the EBC exploit established biochemical methods; three ("*p*-coumaric acid," "Dp 300," and "Plant Indican") are newly developed, nonconventional reagents. A positive control well, containing a nonselective medium, provides a source for subculturing and for additional tests. Manufacturer's instructions were followed throughout the study. After an 8-h incubation, the data were automatically interpreted, and the results were printed. All biochemical reactions were listed along with two organism identifications which most closely fit the reactions and their degree of probability. Reproducibility of the AMS-EBC system results was assessed by running six isolates three times a day for 4 days.

Micro-ID. The Micro-ID system has been described in detail (3). It consists of 15 reaction chambers containing reagent-impregnated paper disks. Each chamber was inoculated with 0.2 ml of a no. 0.5 McFarland standard suspension. Package insert directions were followed in all cases. Results were read after 4 h of incubation, translated into the Micro-ID code, and matched in a computerized manual.

Conventional identification. Each isolate was tested by Micro-ID and AMS. When an identification was not in agreement by the two systems, the isolate was checked for purity and then identified by the standard methodology of Ewing and Martin (6).

RESULTS

Enterobacteriaceae isolates tested with the AMS-EBC are listed in descending order of frequency in Table 1. The majority of clinical isolates were represented by *E. coli* and *K. pneumoniae*; together, these species accounted for 54% of the total. Stock cultures of nine species were also tested since few, if any, of these isolates were recovered from clinical samples during the period of study.

Identification by the AMS-EBC and Micro-ID differed in only 12 of 1,528 isolates (0.8%). All discrepancies were with clinical isolates. When these 12 strains were identified by standard methodology, 2 had been misidentified by both systems, 2 had been misidentified only by Micro-ID, and 8 had been misidentified only by AMS. The total of 10 errors by AMS and their reaction differences with standard methodology are shown in Table 2. Misidentifications by AMS were almost totally confined to tbe *Enterobacter* spp. *Enterobacter cloacae* was misidentified four times as *S. liquefaciens* and two times as *Enterobacter aerogenes*. Inspection of individual biochemical tests performed by AMS revealed that a negative arginine test was the cause in all six instances for the misidentification of *E. cloacae* strains. In four of the isolates misidentified as *S. liquefaciens* the urease was also negative, whereas in the other two misidentifications sorbitol was positive, raising the probability for identification as *E. aerogenes*. However, *E. cloacae* was correctly identified 177 times out of 183 for a 96.7% accuracy rate. A number of tests characteristically positive were negative for *E. aerogenes*, causing it to be misidentified as *Klebsiella ozaenae* with the most important biochemical being ornithine. A negative malonate test coupled with the impossibility for the AMS module to recognize the characteristic yellow pigment of *Enterobacter agglomerans* contributed to the one misidentification of this isolate as *K. ozaenae*. A negative rhamnose test was responsible for the misidentification of *K. pneumoniae* as *Serratia rubidaea*, and a negative urease was responsible for *Proteus rettgeri* being identified as *Providencia stuartii*.

Results of reproducibility testing showed that five of six organisms were always correctly identified. In one instance, *K. pneumoniae* was identified as *S. rubidaea* because of a negative rhamnose test. Thus, only one of 72 tests (1.4%) deviated from the expected.

DISCUSSION

Our results confirm those of Isenberg et al. (7) and show that the AMS-EBC is capable of reliably identifying clinical isolates of the *Enterobacteriaceae* after primary isolation. The frequency of disagreements with the Micro-ID was extremely low, and reproducibility was satisfactory. We chose the Micro-ID for comparison since it is regarded as an accurate means of identifying *Enterobacteriaceae*. In fact, after correction of earlier problems with the Micro-ID identification manual and extensive testing of *Enterobacteriaceae* isolates, reported disagreements with a standard technique are in the 2 to 4% range (2–5). The rationale for using conventional methods only in case of a disagreement between AMS and Micro-ID in this work seems to be strengthened by recent studies comparing two test methods (API 20E and Micro ID) with reference techniques (2, 5). These studies indicate that the probability of two systems misidentifying the same isolate in exactly the same way is extremely low. When directly comparing two test systems, the number of disagreements approximates the sum rather than the difference of the disagreements of each test with the reference techniques (5). Thus, even for methods using a very similar array of biochemical reactions, small differences in media formulation and in the choice of a data base tend to assign to each method unique patterns of misidentification.

A specific pattern of misidentification for AMS-EBC was indeed apparent in this study since 6 out of 10 errors occurred with *E. cloacae*,

TABLE 1. *Comparison of AMS-EBC and Micro-ID for the identification of Enterobacteriaceae*

Bacterium	No. of clinical isolates	No. of stock cultures	No. of total strains tested	Agreement	
				No.	%
Escherichia coli	504		504	503	99.8
Klebsiella pneumoniae	320		320	319	99.7
Enterobacter cloacae	183		183	177	96.7
Proteus mirabilis	168		168	167	99.4
Enterobacter aerogenes	76		76	75	98.7
Serratia marcescens	61		61	61	100
Citrobacter diversus	30		30	30	100
Citrobacter freundii	31		31	31	100
Morganella morganii	30		30	30	100
Enterobacter agglomerans	15		15	14	93.3
Proteus vulgaris	15		15	15	100
Proteus rettgeri	15		15	14	93.3
Serratia liquefaciens	14		14	14	100
Hafnia alvei	8	4	12	12	100
Providencia stuartii	7		7	7	100
Providencia alcalifaciens	7		7	7	100
Salmonella species	1	6	7	7	100
Arizona hinshawii		6	6	6	100
Shigella species		6	6	6	100
Edwardsiella tarda		6	6	65	100
Klebsiella ozaenae	2	3	5	4	100
Klebsiella rhinoscleromatis		4	4	3	100
Serratia rubidaea		3	3	3	100
Yersinia enterocolitica		3	3		
Total	1,487	41	1,528	1,516	99.2

TABLE 2. *AMS-EBC misidentifications*

No. of isolates	Standard identification	AMS-EBC identification	Reaction discrepancy[a]
4	*E. cloacae*	*S. liquifaciens*	ARG−, URE−
2	*E. cloacae*	*E. aerogenes*	ARG−, SOR+
1	*E. aerogenes*	*K. ozaenae*	ORN−, LAC−, CIT−, RHA−, MLT−
1	*E. agglomerans*	*K. ozaenae*	MAL−, SUC−, INO−, MLT−
1	*K. pneumoniae*	*S. rubidaea*	RHA−
1	*P. rettgeri*	*P. stuartii*	URE−

Abbreviations: ARG, arginine; URE, urease; SOR, sorbitol; ORN, ornithine; LAC, lactose; CIT, citrate; RHA, rhamnose; MLT, maltose; MAL, malonate; SUC, sucrose; INO, inositol.

a common isolate. A false-negative arginine test was responsible for these misidentifications. Although some strains of *E. cloacae* are known to yield a slow arginine reaction, the biochemical is important in differentiating *E. cloacae* from several other genera and species. In the Isenberg et al. collaborative study, 1 out of 10 strains of *E. cloacae* was misidentified as *Yersinia enterocolitica* in all six laboratories, and this was primarily due to a negative arginine test (7). The cause was attributed to a particular EBC lot since retesting with a different lot produced correct reactions. We obtained 96.7% accuracy (177 out of 183 strains), indicating that the vast majority of clinical isolates produce enough arginine dihydrolyase to be correctly identified. However, since arginine did not cause a particular problem with any other organism, possible misidentification of *E. cloacae* must be considered, especially in view of the high incidence of this organism in clinical isolates. Perhaps the problem might be corrected by automatic lowering of the arginine threshold level when other reactions are compatible with *E. cloacae*. A false-negative citrate reaction was an important contributing factor in the majority of misidentifications in the Isenberg et al. study. The interpretation was that a low or improperly prepared inoculum or slow citrate utilization was the probable cause. We did not experience a high incidence of false-negative citrate reactions, and citrate utilization did not present a critical problem with our clinical isolates. As in commercial systems, lot-to-lot variability in the media may account for small differences in concentrations

of key biochemicals and may be responsible for slight differences resulting in discrepancies with false-positive or false-negative reactions. We agree with Isenberg et al., however, that as more strains or organisms which produce various amounts of specific enzymes are assayed, the substrate concentration, basal media, and threshold settings on the instrument may need to be adjusted.

Less common *Enterobacteriaceae*, whose final identification may be time consuming with conventional techniques, were accurately characterized in all instances. Once the reliability of a system has been proven, other factors such as time and cost should be considered in evaluating the performance of the system in the clinical laboratory. The time required for inoculation of the AMS-EBC is approximately the same as that for the Micro-ID. The total incubation time is 8 h for the AMS-EBC and 2 h for Micro-ID. In the vast majority of the cases it was possible to inoculate both systems on the first day from primary plates. With the AMS, no time is needed for recording and interpreting the reactions. The AMS computer can also be directly connected to a hospital or a laboratory computer, thus saving the time employed in transferring and reporting results. The high initial cost of the AMS could be balanced by fully exploiting its versatility. Several other programs including susceptibility testing and direct identification of important urine isolates are available. The new EBC plus card, which includes the identification of certain nonfermenting gram-negative bacilli, is now available. Preliminary data in our laboratory indicate a high reliability of the expanded system.

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