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Evaluation of the autoSCAN-W/A Rapid System for Identification and Susceptibility Testing of Gram-Negative Fermentative Bacilli

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The autoSCAN-Walk-Away (W/A) system for identification and susceptibility testing was evaluated for 400 gram-negative fermentative bacteria by using the API 20E (366 isolates) and/or tube biochemical tests as the reference identification system and a frozen microdilution MIC tray system for susceptibility testing. The W/A system performed well for identification of this group of organisms representing 14 genera and 30 species, showing a sensitivity of 96% and results available in 2 h. Of the 16 misidentifications, 6 were with *Serratia liquefaciens*. A total of 63 isolates (17%) required further tests to complete the identification, compared with 106 (29%) of the isolates which required additional tests for the API 20E identification. Approximately half (32) of the additional tests with the W/A system were required in order to separate *Citrobacter diversus* from *C. amalonaticus*. For susceptibility determinations, the W/A system demonstrated an overall agreement of 93% (4,102 determinations) with 40 major errors (0.98%). However, of the 906 resistant organism-drug combinations in the study, there were 115 very major errors, for a false-susceptibility rate of 12.7% of the resistance determinations. Among these very major errors, 80% occurred with piperacillin and the cephalosporins. The W/A system completed the MIC determinations in 7 h; however, the difficulty in detecting resistance with some antimicrobial agents limited the advantages of the rapid susceptibility testing.

The contribution of the clinical microbiology laboratory in the effective diagnosis and treatment of bacterial infections depends on timely identification and susceptibility testing of bacteria (3, 14, 15). Truly rapid identification and susceptibility tests of gram-negative bacilli can have a significant impact on the management of infections, especially those infections caused by newly emerging antibiotic-resistant bacteria.

Classically, the identification of these gram-negative bacilli has been performed by detecting their utilization of different substrates as sources of carbon and nitrogen through the use of conventional tube biochemical tests (7). This method is slow, expensive, and cumbersome (1) and has been replaced by commercial systems, such as the API 20E system (Analytab Products, Plainview, N.Y.). The API 20E system contains substrates similar to the conventional tube biochemicals in a microcupule format coupled with a computer-assisted biocode system. This system is accurate and cost-effective and has become a standard with which newer tests are compared (6, 19). Susceptibility testing of gram-negative bacilli to determine MICs has progressed from tube macrodilution tests to microdilution tests, which have become readily available from commercial sources (12). The API identification system and a microdilution MIC system are convenient and accurate; however, they do not provide same-day results with minimal hands-on time of the technologist (15). A few systems which do provide results in 5 to 8 h by either manual, semiautomated, or automated methods have emerged (4, 25).

The fully automated autoSCAN-Walk-Away (W/A) system (Baxter Diagnostics, West Sacramento, Calif.) provides rapid identification of gram-negative bacilli in 2 h and susceptibility test results in 7 h by using lyophilized trays containing fluorogenic substrates and pH indicators. The resulting fluorescence is detected with 10 to 100 times more sensitivity than colorimetric detection provides. Several evaluations of the rapid W/A system have been presented in abstract form (2, 5, 11, 22), but there have been few detailed reports of the capability of the W/A system for identification of gram-negative bacilli (20) or susceptibility testing (10). We report a study of the W/A system's ability to perform rapid identification and susceptibility determinations in comparison with the API 20E system and conventional methods for identification and a microdilution MIC method for susceptibility testing.

MATERIALS AND METHODS

Test organisms. Isolates of glucose-fermenting gram-negative rods from 14 genera representing 30 species commonly encountered in clinical laboratories were selected. Each genus was equally represented when possible. In order to challenge the rapid W/A system, organisms which presented potential identification problems or were highly antibiotic resistant were selected. A total of 400 isolates from the family *Enterobacteriaceae* and the genus *Aeromonas* were tested; the isolates were either recent clinical isolates or from frozen stock collections. Isolates from the frozen collection were first subcultured twice on sheep blood agar and incubated for 18 to 24 h in ambient air at 35°C. All 400 isolates were subcultured on MacConkey agar and incubated for 18 to 24 h at 35°C in ambient air prior to testing on the autoSCAN-W/A.

Reference identification method. The reference identification of the isolates was determined by one of three methods: (i) the API 20E method with additional tests, as required by the instructions; (ii) rapid indole production, swarming on

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blood agar, and ornithine decarboxylase for *Proteus* spp.; or (iii) rapid indole production, hemolysis on sheep blood agar, lactose fermentation on MacConkey agar, and hydrolysis of 4-methylumbelliferyl β-D-glucuronide (Sigma Chemical Co., St. Louis, Mo.) for *Escherichia coli* (27).

If the identification obtained by the W/A system did not agree with the reference identification, both methods were repeated with the same subculture of the organism. This repeat was expected to pick up discrepancies caused by storage. All discrepancies after repeated testing were resolved by additional conventional tube biochemical tests (8, 9, 26).

The autoSCAN-W/A system. The autoSCAN-W/A system consisted of the autoSCAN-W/A, a complete panel incubation-interpretation system, and an IBM Personal System/2 computer using MicroScan DMS version 17.02 software. The W/A system automatically made fluorometric readings and interpreted the biochemical patterns and MICs. Each inoculum was prepared by suspending enough colonies from a MacConkey agar plate into 6.5 ml of 0.4% saline–Pluronic water (BASF Wyandotte, Wyandotte, Mich.) to achieve a density equivalent to a 0.5 McFarland standard; 0.1 ml of this suspension was inoculated into 25 ml of cation-supplemented Mueller-Hinton broth. By using a RENOK inoculator, the inoculated cation-supplemented Mueller-Hinton broth was dispensed into the MIC portion of a Rapid Negative Combo Type 2 panel (supplied by the manufacturer), and the saline-Pluronic water was dispensed into the identification portion of the panel. Each of the wells containing decarboxylase tests was covered with 3 drops of mineral oil. A 0.001-ml sample from the Mueller-Hinton broth was streaked onto blood agar to determine purity and colony count. Tests were accepted if more than 100 CFU was present after overnight incubation at 35°C. Readings for identification of the isolates were obtained at 40 min and finally at 2 h; the 2-h reading was accepted if the probability was ≥85%. Otherwise, additional tests displayed by the W/A computer terminal were performed and matched to one of the species designated. If the W/A identified the organism to the genus level only, except for *Salmonella* and *Kluyvera* spp., additional biochemical tests, including the spot indole test and tube biochemical tests, were performed to determine the species, as appropriate.

API biochemical identification. The API 20E strips were inoculated, incubated for 18 to 24 h, and interpreted according to the manufacturer's instructions to determine a biocode. An identification was accepted if the organism's biocode as listed in the Analytical Profile Index was either excellent, very good, or acceptable or if the additional tests required by the index for a low-selectivity identification matched with one of the species alternatives listed. Similarly, the API computer-assisted telephone service was used to determine an acceptable identification if a biocode was not found in the profile index. If the API identified the organism to the genus level only, additional biochemical tests were performed to determine the species, either as directed by the index or by using conventional identification charts (8, 9, 26). *Salmonella* and *Kluyvera* spp. were not further identified if the API did not do so.

Susceptibility testing. The reference microdilution susceptibility tests were performed with microdilution trays prepared in-house according to procedures of the National Committee for Clinical Laboratory Standards (NCCLS) (18) and frozen at −70°C until use. Each inoculum was prepared by growing the organism in brain heart infusion broth for several hours, diluting the inoculum in 0.02% Tween 80 in sterile water to a final concentration of 10^7 to 10^8 CFU/ml, and inoculating the trays with a MIC 2000 automatic inoculator (Dynatech Laboratories, Inc., Alexandria, Va.) to yield a final concentration of approximately 5×10^5 CFU/ml. A 0.001-ml sample from the growth control well was streaked on blood agar to verify the purity and colony count of the inoculum. Counts were accepted if more than 100 CFU was present after overnight incubation. The inoculated trays were covered with tape (Dynatech) and incubated in ambient air at 35°C for 16 to 20 h. The MICs were determined by observing the presence or absence of visible growth under transmitted light.

During the study period, the drugs present in the prepared MIC trays varied depending on whether the isolate was from urine, stool, or other sources. In addition, trays used in the later part of the study did not contain cefotaxime. Hence, the total number of determinations varied for each antimicrobial agent tested.

Quality control. The quality control organisms *E. coli* ATCC 25922 and *Klebsiella oxytoca* AmMS 101 were tested daily with the W/A system. In addition, the quality control organisms *Aeromonas hydrophila* AmMS 199, *Pseudomonas putrefaciens* AmMS 201, and *Acinetobacter anitratus* AmMS 202 were tested initially and with each new lot of W/A panels. If out-of-range endpoints were obtained, the testing was repeated. Each lot of API strips was tested with *Klebsiella pneumoniae* ATCC 13883, *Enterobacter cloacae* ATCC 13047, *Proteus vulgaris* ATCC 13315, and *Pseudomonas aeruginosa* ATCC 10145 to provide a positive and a negative reaction for each biochemical test in the profile. For the reference MIC method, the quality control organisms *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested weekly and with each new lot of trays (18).

Interpretation of data. The W/A rapid identification was considered in agreement with one of the reference methods if it yielded the same organism identification with or without the additional tests required by the W/A system's instructions. If the W/A and the reference methods disagreed after repeated testing, then the identification with additional conventional biochemical tests was defined as the correct identification against which the other methods were evaluated for agreement. In our evaluation, neither the spot indole test nor the spot oxidase test was considered an additional test, since these tests are simple and rapid; the spot oxidase test is part of the API 20E test battery.

All susceptibility interpretations were in accordance with those recommended by NCCLS (18). If the susceptibilities for an isolate disagreed by more than one dilution for three or more drugs tested, the susceptibility tests were repeated by both methods with the same subculture, and the susceptibilities obtained in the repeated tests were used in the comparison. Essential agreement was met if the W/A MIC endpoint for each drug was within one dilution of the microdilution MIC endpoint. Errors were defined as follows. A very major error was a result in which the W/A method categorized the isolate as susceptible to an antimicrobial agent and the microdilution method categorized the isolate as resistant. A major error was a result in which the W/A method categorized the isolate as resistant and the microdilution method categorized the isolate as susceptible. The percentages of very major errors and major errors were defined as 100 times the number of these errors divided by the total number of determinations (24). In addition, the number of very major errors was compared with the total number of determinations of resistance.

TABLE 1. Identification of isolates by the autoSCAN-W/A and API 20E systems

Species	No. of isolates tested	No. incorrectly identified		No. requiring additional tests	
		W/A	API	W/A	API
Aeromonas caviae	17	0	0	0	17
Aeromonas hydrophila and *A. sobria*	19	0	0	0	0
Citrobacter amalonaticus	15	2	0	13	15
Citrobacter diversus	19	0	0	19	18
Citrobacter freundii	21	1	0	4	7
Edwardsiella tarda	5	0	0	0	0
Enterobacter aerogenes	10	0	1	2	2
Enterobacter agglomerans	8	3	0	3	2
Enterobacter asburiae	8	1	1	1	2
Enterobacter cloacae	31	0	3	7	6
Escherichia coli	18	0	0	0	1
Hafnia alvei	7	0	0	0	2
Klebsiella oxytoca	23	0	0	0	1
Klebsiella pneumoniae	29	0	1	0	4
Klebsiella rhinoscleromatis	2	2	0	2	0
Kluyvera spp.	6	1	0	2	1
Morganella morganii	18	0	0	0	1
Proteus mirabilis	10	0	0	2	0
Proteus penneri	11	0	0	1	0
Proteus vulgaris	1	0	0	0	0
Providencia alcalifaciens	3	0	0	0	0
Providencia rettgeri	2	0	0	1	0
Providencia stuartii	7	0	2	0	1
Salmonella spp.	21	0	1	2	7
Serratia liquefaciens	12	6	0	1	7
Serratia marcescens	18	0	0	0	3
Serratia odorifera	1	0	0	0	0
Serratia rubidaea	3	0	0	1	0
Shigella flexneri	11	0	0	2	9
Shigella sonnei	10	0	0	0	0
Total	366	16	9	63	106

TABLE 2. Incorrect identifications by the W/A and API systems

Incorrect identification (no. of isolates)	Correct identification (no. of isolates)
W/A	
Enterobacter amnigenus (1)	
Escherichia coli (1)	*Citrobacter amalonaticus* (2)
Enterobacter cloacae (1)	*Citrobacter freundii* (1)
Klebsiella pneumoniae (2)	
Enterobacter cloacae (1)	*Enterobacter agglomerans* (3)
Enterobacter cloacae (1)	*Enterobacter asburiae* (1)
Enterobacter aerogenes (2)	*Klebsiella rhinoscleromatis* (2)
Klebsiella sp. (1)	*Kluyvera* sp. (1)
Serratia marcescens (5)	
Ewingella americana (1)	*Serratia liquefaciens* (6)
API 20E	
Serratia liquefaciens (1)	*Enterobacter aerogenes* (1)
Enterobacter intermedium (1)	*Enterobacter asburiae* (1)
Citrobacter amalonaticus (1)	
Citrobacter freundii (1)	
Serratia liquefaciens (1)	*Enterobacter cloacae* (3)
Enterobacter agglomerans (1)	*Klebsiella pneumoniae* (1)
Providencia alcalifaciens (2)	*Providencia stuartii* (2)
Salmonella paratyphi A (1)	*Salmonella heidelberg* (1)

RESULTS

Biochemical identification. A total of 400 isolates of gram-negative bacilli were tested; of these, the W/A rapid system correctly identified 384 isolates (96.0%). Of the 366 isolates tested by the API 20E and W/A systems, the W/A system correctly identified 350 isolates (95.6%) and the API 20E system correctly identified 357 isolates (97.5%) (Table 1). The W/A system correctly identified, without additional tests, the 34 isolates that had been identified by reference methods other than the API 20E method; these isolates consisted of 18 *E. coli*, 11 *Proteus mirabilis*, and 5 *Proteus vulgaris* isolates.

The 16 W/A misidentifications were contained within 5 of the 14 genera tested; 9 were misidentifications at the genus level and 7 were incorrect at the species level (Table 2). The API misidentifications were contained within 4 of the 14 genera. Of the nine misidentifications, five were incorrect at the genus level and four were incorrect at the species level. In no case were the API and W/A identifications simultaneously found to be incorrect when conventional tests were required to resolve a discrepancy. The W/A system required additional biochemical testing for 63 isolates (17%), and the API system required further tests for 106 isolates (29%).

The W/A system misidentified 2 isolates of *Klebsiella rhinoscleromatis* as *Enterobacter aerogenes*, 3 of the 8 *Enterobacter agglomerans* isolates, and 6 of the 12 *Serratia liquefaciens* isolates. The API system identified all of the *Serratia* isolates correctly. However, 7 of the 12 *Serratia liquefaciens* isolates were identified to the genus level only and required xylose and raffinose fermentation to complete the identification to the species level.

Neither the W/A system nor the API system identified to the species level the 36 *Aeromonas* isolates in the study; rather, the designation *A. hydrophila* group was utilized. Both systems correctly identified *A. hydrophila* and *Aeromonas sobria* to the genus level, but the API system required growth inhibition on 5% salt agar as an additional test to identify the 17 *Aeromonas caviae* isolates correctly to the genus level and to differentiate them from *Vibrio fluvialis*. Consequently, the 17 salt tolerance tests were counted as additional tests for the API system.

The W/A system required additional tests to correctly identify 4 of the 55 *Citrobacter* isolates; however, *Citrobacter amalonaticus* and *Citrobacter diversus* were classified as belonging to the *C. amalonaticus-C. diversus* group by the W/A system. In order to separate these isolates to the species level, at least one of the following tests was done: malonate, adonitol, or KCN. Thus, a total of 32 isolates required additional tests in order to identify *C. diversus* and *C. amalonaticus* to the species level. The API system required additional tests for 13 *Citrobacter* isolates because of low selectivity and for 27 isolates to identify them to the species level.

As for the 57 *Enterobacter* isolates in the study, the W/A system required additional tests for 13 isolates because of low probability and the API system required additional tests for 12 isolates. None of the *Klebsiella* isolates required additional tests by the W/A system, but five *Klebsiella* isolates required additional tests with the API 20E system because of low selectivity. The W/A system did not separate *K. oxytoca* from *K. pneumoniae* or *Proteus penneri* from *Proteus vulgaris*, but this was easily done by performing a spot indole test. The W/A system required further testing only for two *Salmonella* and two *Shigella flexneri* isolates, while the API required additional tests for seven *Salmonella* and nine *Shigella flexneri* isolates because of low selectivity. Neither system required extra tests for *Shigella sonnei*.

TABLE 3. W/A system drug MIC ranges and expected endpoints for quality control strains

Antimicrobial agent	MIC or MIC range (mg/liter)		
	W/A Rapid Negative Combo Type 2	*E. coli* ATCC 25922[a]	*K. oxytoca* AmMS 101
Ampicillin	2–16	≤2–4	≥16
Piperacillin	8–64	<8	≤8–16
Cefazolin	2–16	≤2	4–16
Cefotetan	4–32	<4	≤4
Ceftriaxone	4–32	<4	≤4
Ceftazidime	2–16	<2	≤2
Cefotaxime	4–32	<4	≤4
Gentamicin	1–4, 6	≤1	≤1
Tobramycin	1–4, 6	≤1	≤1
Ciprofloxacin	1–2	<1	≤1
Imipenem	4–8	<4	≤4
Trimethoprim-sulfamethoxazole	0.5/9.5–2/38	≤0.5/9.5	≤0.5/9.5

[a] If the MIC range from NCCLS (18) was entirely below the range tested in the W/A Rapid Negative Combo Type 2 tray, the value is listed as < rather than ≤.

Susceptibility testing. A list of the antimicrobial agents evaluated and the range of dilutions tested by the W/A system is presented in Table 3. The two organisms recommended by the manufacturer for quality control of the MIC testing, *E. coli* ATCC 25922 and *K. oxytoca* AmMS 101, did not have endpoint ranges which could accurately measure the potencies of 10 of the 12 antimicrobial agents evaluated in the panel, since their MICs were equal to or below the lowest concentration of the antimicrobial agent in the wells (Table 3).

The overall essential agreement between the W/A method and the microdilution method was 92.7% (Table 4); 4.7% of the results were 2 or more dilutions lower and 2.6% were 2 or more dilutions higher than the reference MICs. The software of the W/A system suppressed results from certain organism-drug combinations as unreliable by the rapid method because these combinations yielded less than 90% essential agreement when compared with an overnight reference method. The suppressed combinations are indicated in Table 5. We demonstrated less than 90% essential agreement for piperacillin, cefazolin, cefotetan, and ceftriaxone, although results for only one of these drugs (cefazolin) were suppressed for some organism-drug combinations.

TABLE 4. Errors and essential agreement for MIC determinations by the W/A system compared with the microdilution method

Drug	No. of isolates tested	No. of major errors	No. of very major errors (%)	Total essential agreement (%)
Ampicillin	383	1	6 (1.6)	367 (96)
Piperacillin	359	9	11 (3.1)	315 (88)
Cefazolin	361	2	32 (8.9)	306 (85)
Cefotetan	309	0	9 (2.9)	273 (88)
Ceftriaxone	360	1	19 (5.3)	314 (87)
Ceftazidime	360	9	15 (4.2)	330 (92)
Cefotaxime	147	2	5 (3.4)	133 (91)
Gentamicin	384	0	1 (0.3)	364 (95)
Tobramycin	361	0	9 (2.5)	342 (95)
Ciprofloxacin	381	0	0 (0.0)	381 (100)
Imipenem	313	7	1 (0.3)	306 (98)
Trimethoprim-sulfamethoxazole	384	9	7 (1.8)	372 (97)
Total	4,102	40	115 (2.8)	3,803 (93)

Without exclusion of the results of the suppressed organism-drug combinations, there were 906 MIC determinations of resistance. Of these, 658 were classified as resistant by the W/A system, and an additional 133 were classified as moderately susceptible, leaving 115 very major discrepancies. The very major errors represented 12.7% of the 906 resistant organism-drug combinations, or 2.8% of the total determinations in the study. The very major discrepancies were widely distributed among the drugs tested (Table 5); however, cefazolin, cefotetan, ceftazidime, cefotaxime, and ceftriaxone accounted for 70% of the total discrepancies, and piperacillin accounted for 10% of the total.

After the results from organism-drug combinations suppressed by the W/A system were excluded, there were 708 MIC determinations of resistance. Of these, 517 were called resistant by the W/A system and an additional 93 were called moderately susceptible, leaving 98 very major discrepancies. The very major discrepancies represented 13.8% of the 708 resistant organism-drug combinations or 2.6% of the total number of determinations in the study.

The W/A system effectively classified truly susceptible strains, having only 40 major errors among the 4102 determinations (0.98%). These errors were seen almost exclusively with the *Serratia* (18 errors) and *Proteus-Morganella* (11 errors) isolates. If the results of the suppressed organism-drug combinations were excluded, there were 37 major discrepancies (0.98%) among 3,787 determinations.

Time required for completed results. The panels in the W/A system were automatically read hourly until completion. Although the identification was completed in 2 h, it could take up to 7 h for the completion of the MIC results. In this study, the cumulative percentage of tests completed was 16.2% after 3.5 h, 83.5% after 4.5 h, 98.1% after 5.5 h, and 100% after 7 h.

DISCUSSION

The ability of the W/A system to identify enteric gram-negative rods accurately in 2 h is a major attraction of this automated system. The W/A system's ability to identify members of the *Enterobacteriaceae* in a rapid and accurate manner has been demonstrated in a number of laboratories. In a multicenter trial, the W/A system correctly identified 96.4% of 753 clinical isolates compared with an overnight microdilution method (5). Comparison of the W/A system and the AutoMicrobic System (Vitek AMS; Vitek Systems, Inc., Hazelwood, Mo.) showed that the W/A system correctly identified 95% of the tested strains (11, 20). Colonna et al. (2) compared the W/A system with the API system and found 83% agreement for 358 strains, with an additional 7.8% of results being inconclusive. In the present study, the W/A system correctly identified 96% of 400 isolates, indicating that it is an acceptable, accurate identification system (24).

Previous reports indicated that 5 to 9% of the enteric bacteria required additional tests for identification (2, 5, 20), increasing the time required for identification from 2 to 24 or 48 h for some isolates. We found that 8.5% of the isolates required additional tests in the W/A system if the *C. amalonaticus-C. diversus* group and the *A. hydrophila* group were not further separated; however, when the *Citrobacter* spp. were included, 17% of the isolates tested with the W/A

TABLE 5. Detection of antimicrobial resistance by the W/A system in comparison with the reference MIC method

Species[a]	Result for indicated antibiotic[b]											
	AM	PI	CFZ	CTN	CAX	CAZ	CFT	GM	TO	CP	IMP	T-S
Aeromonas spp. (36)												
Total no. resistant	36[c]	5	22[c]	5	2	2	1	1	1	0	1	3
Very major errors	0	1	6	3	0	1	0	0	0	0	1	0
Citrobacter diversus-C. amalonaticus (33)												
Total no. resistant	33	13	16[c]	0	5	0	0	4	3	1	0	2
Very major errors	1	3	0	0	5	0	0	0	1	0	0	0
C. freundii (21)												
Total no. resistant	16[c]	13	19[c]	7	10	11	7	7	7	0	0	3
Very major errors	1	0	3	2	1	3	1	0	0	0	0	1
Escherichia coli (36)												
Total no. resistant	18	17	4	0	0	1	0	6	4	0	0	13
Very major errors	0	3	2	0	0	0	0	0	2	0	0	0
Enterobacter aerogenes (10)												
Total no. resistant	9	7	9	6	5	6	2	0	0	0	0	0
Very major errors	0	1	3	1	3	2	2	0	0	0	0	0
E. agglomerans (7)												
Total no. resistant	4	1	2	0	0	0	0	1	1	0	0	0
Very major errors	0	0	0	0	0	0	0	0	0	0	0	0
E. asburiae (8)												
Total no. resistant	8[c]	3	8[c]	3	5	5	2	0	0	0	0	0
Very major errors	4	0	1	0	1	1	1	0	0	0	0	0
E. cloacae (28)												
Total no. resistant	28[c]	15	28[c]	22	15	20	9	3	4	0	0	2
Very major errors	0	0	0	2	1	5	0	0	1	0	0	2
Hafnia alvei (7)												
Total no. resistant	7	2	6	1	2	3	1	0	0	0	0	0
Very major errors	0	0	0	0	0	1	0	0	0	0	0	0
Klebsiella spp. (52)												
Total no. resistant	50	10	18	0	0	0	0	5	5	1	0	2
Very major errors	0	0	11	0	0	0	0	0	0	0	0	1
Kluyvera spp. (6)												
Total no. resistant	4	0	4	0	0	0	0	0	0	0	0	0
Very major errors	0	0	1	0	0	0	0	0	0	0	0	0
Morganella morganii (17)												
Total no. resistant	17	3	17	3	1	5	2	1	0	0	0	2
Very major errors	0	0	0	0	1	2	1	1	0	0	0	0
Providencia spp. (12)												
Total no. resistant	8[c]	2	9[c]	0	0	0	0	1	0	0	0	8
Very major errors	0	0	4	0	0	0	0	0	0	0	0	0
Proteus mirabilis (21)												
Total no. resistant	3	2	2	0	0	0[c]	0	4	4	0	0	0
Very major errors	0	2	0	0	0	0	0	0	3	0	0	0
P. penneri (10)												
Total no. resistant	10[c]	1	10[c]	0	6	0	0	1	1	0	0	3
Very major errors	0	1	0	0	6	0	0	0	1	0	0	1
P. vulgaris (6)												
Total no. resistant	6	0	6[c]	0	1	0	0	0	0	0	0	0
Very major errors	0	0	0	0	1	0	0	0	0	0	0	0
Salmonella spp. (21)												
Total no. resistant	5	4	0	0	0	0	0	2[c]	2[c]	0	0	2
Very major errors	0	0	0	0	0	0	0	0	1	0	0	0
Serratia spp. (33)												
Total no. resistant	22	2	31	1	0	0	0	1	2	0	0	0
Very major errors	0	0	1	1	0	0	0	0	0	0	0	0
Shigella spp. (21)												
Total no. resistant	13							0[c]		0		7
Very major errors	0							0		0		2
Total no. resistant	297	100	211	48	52	53	24	37	34	2	1	47
Total no. of very major errors	6	11	32	9	19	15	5	1	9	0	1	7

[a] Numbers in parentheses are total numbers of isolates tested. No resistance was seen with the four isolates of *Edwardsiella tarda*.

[b] AM, ampicillin; PI, piperacillin; CFZ, cefazolin; CTN, cefotetan; CAX, ceftriaxone; CAZ, ceftazidime; CFT, cefotaxime; GM, gentamicin; TO, tobramycin; CP, ciprofloxacin; IMP, imipenem; T-S, trimethoprim-sulfamethoxazole.

[c] Results suppressed by the W/A system.

system required additional tests. In contrast, the API 20E system is already an 18- to 24-h test, and for the 29% of the isolates that required additional tests, the time to identification was increased to 48 to 72 h. Since the W/A system requires MacConkey agar for initial growth of the isolate and requires several colonies to provide enough inoculum for the test, the actual time from colony detection until the test can be performed in a clinical setting may actually be extended by 1 day for some isolates. The API test can be performed with one colony of an organism growing on any type of medium or even with fluid from positive blood cultures (17).

We assumed that the API system had the correct identification as long as the W/A system was in agreement with it. Only when the two disagreed were conventional biochemicals used. Thus, there may have been some cases of misidentification of isolates by both systems that were not detected by the test protocol. However, O'Hara et al. (19) recently reevaluated the API 20E system in comparison with conventional tests and found that 95.2% of 291 isolates of members of the *Enterobacteriaceae* were correctly identified after the additional biochemical tests recommended by the manufacturer. In our study, the API result was considered to be correct for 97.5% of the 366 isolates tested. Thus, our assumption that the API identification was correct is reasonable and should not affect the overall results.

The susceptibility testing component of the W/A system appeared to have some limitations. We found 93% essential agreement with the reference system, which was similar to that reported in other studies (10, 22). Godsey et al. (10) evaluated seven antimicrobial agents with 1.5% very major errors, and Sahm et al. (22) evaluated the W/A system with the Vitek AMS system for 292 isolates and 4,964 determinations with 2.7% very major errors. Neither of these studies indicated the prevalence of resistant organism-drug determinations in their test populations. With 22% of the determinations in our study categorized as resistant by the microdilution method, we showed a very major error rate of 2.6 to 2.8%. Such errors are most serious; Sherris and Ryan (24) caution that very major errors should not exceed 1.5% of the total determinations for any antimicrobial agent reported. In this study, piperacillin, tobramycin, and each of the five cephalosporins had very major error rates in excess of the recommended 1.5% (Table 4).

The performance of the W/A system in detecting resistance was not surprising. A rapid test for detection of the susceptibility of an organism to a given antimicrobial agent is more apt than a conventional test to err on the side of calling an organism susceptible when it is truly resistant, since the organism is required to register some growth signal in a shorter time than would be required in an overnight assay (13). For most drugs, an organism must be actively growing in order to produce amounts of enzymes sufficient to inactivate or otherwise resist the antimicrobial agent. If the organism has difficulty growing or requires several generations to develop its enzymes or alternate pathways of resistance, it will be considered susceptible to the drug when it is truly resistant. We chose for inclusion in the study strains that challenged the ability of the W/A system to detect resistance. If very few resistant isolates were included in the study, then the W/A system would have compared favorably with the microdilution method, even though it may really do poorly in detecting resistance. We recommend that, to make meaningful use of the data, published evaluations of rapid susceptibility test systems report the number of resistant interpretative categories that were included in the study.

Miller (16) has suggested that laboratories develop standards for the comparison of one commercial identification system with another. The same standards need to be developed in evaluating MIC determinations. For example, the classical definition of the percentage of very major errors uses the total number of MIC determinations as the denominator (24). Such a percentage is misleading because very major errors can occur only for determinations in which the reference method has an interpretation of resistant. To use the total number of determinations could mask the true ability of the system to detect resistance. When the total number of determinations in the resistant interpretive category was used as the denominator in the present study, there were 12.6% very major errors among the resistant isolates and drugs tested (13.8% after exclusion of the suppressed combinations). These high percentages greatly exceed the current requirements for automated susceptibility instrumentation. However, any laboratory could calculate from this percentage the number of very major errors that might be expected for the W/A system within its hospital's unique organism antibiogram. In fact, the data presented in Table 5 can be used to calculate the potential error rate for each organism-drug combination, keeping in mind that strain variability does exist in different geographic areas.

We saw no problem with the W/A system's suppression of selected susceptibility results, because these results were for drugs not commonly used to treat the indicated organisms. However, there were problems with some organism-drug combinations that were not suppressed. Of the 18 *Klebsiella* isolates that were resistant to cefazolin, 11 were characterized as susceptible by the W/A system. Since *Klebsiella* infections are often treated with cefazolin, detection of resistance to this agent is important. Unfortunately, we did not test any *Klebsiella* isolates which have acquired resistance to broad-spectrum cephalosporins. The W/A system did not accurately detect resistance to narrow-spectrum cephalosporins among *Klebsiella* isolates, suggesting that further studies are needed to determine whether the W/A system can reliably detect the newly emerging resistance of klebsiellas to broad-spectrum cephalosporins (21).

Among the expanded- and broad-spectrum cephalosporins tested, the W/A system was able to detect only 73% of the 177 resistant combinations. *Citrobacter*, *Enterobacter*, *Morganella*, and *Serratia* isolates and the indole-positive *Proteus* isolates accounted for most of the false-susceptible results, possibly because they possess class I inducible β-lactamases (23). The inducible enzymes probably were not expressed in the short incubation time of the W/A testing. Use of a broad-spectrum cephalosporin can be an acceptable treatment for infections caused by these organisms. Consequently, it is important that a laboratory accurately report these susceptibilities.

The W/A system effectively detected resistance to ampicillin. All of the very major errors were with the *Citrobacter* and *Enterobacter* species, which are predictably resistant to ampicillin. The essential agreement with piperacillin was 88%; both major and very major errors were seen, and, unfortunately, most errors were made for genera for which piperacillin might be a drug of choice. The two isolates resistant to ciprofloxacin were detected by the W/A system, but the single isolate resistant to imipenem was not detected. With the exception of *Xanthomonas maltophilia* and *Pseudomonas aeruginosa*, two species whose testing is problematic for the W/A system, very few imipenem-resistant strains were found. More resistant strains need to be tested

before we can be confident that the W/A system detects resistance to imipenem or ciprofloxacin.

The ideal reference strains for quality control require MICs that fall near the middle of the range of concentrations of the agents tested (18). Only the MIC of cefazolin, with *K. oxytoca* AmMS 101 as a control, fulfilled this requirement. An alternative is to use one control strain for which the modal MIC is equal to or no less than one doubling dilution less than the lowest concentration tested and another strain for which the modal MIC is equal to or no greater than one doubling dilution higher than the highest concentration tested (18). The combination of the *E. coli* and *K. oxytoca* control organisms may fulfill the alternative control guidelines for ampicillin if the modal MIC for *K. oxytoca* is not greater than 32 mg/liter. These two organisms were not adequate controls for the other 10 drugs evaluated in this study. Since pseudomonads often do not grow well in the 7-h incubation needed for the W/A test, *Pseudomonas aeruginosa* ATCC 27853, which is routinely used in MIC quality control, cannot be used with the W/A system. If *A. anitratus* AmMS 202 is tested as a third control agent, five other antimicrobial agents might have controls, depending on their modal MICs. Even so, five antimicrobial agents are clearly without an adequate control. The lack of reliable controls is a serious problem with the W/A system and raises a question about the accuracy of the results for the test organisms. If more control strains are used, the results will be more reliable, but the cost of quality control will be high. Since imipenem is among the most labile agents, it is particularly important that a strain be found to adequately act as a control for this antimicrobial agent. Seven isolates which were characterized as susceptible by the microdilution method were characterized as resistant by the W/A system. These seven major errors could have been due to deterioration of the drug in the wells.

In summary, the ability of the W/A system to report identifications in as little as 2 h makes it the fastest identification system of the rapid systems currently available. It was accurate and required less additional testing than the API 20E. For these reasons, the W/A system is a system which produces bacterial identifications in a clinically useful time frame. However, the ability of the system to detect resistance to many antimicrobial agents, especially the cephalosporins and piperacillin, is limited and will require further modifications to make it optimal. Considering the benefits of rapid reporting of results, the very major errors that occur may be acceptable, depending on the frequency of antimicrobial resistance in a given institution and the seriousness of a very major error in a specific patient population.

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