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Potential Impact of the VITEK 2 System and the Advanced Expert System on the Clinical Laboratory of a University-Based Hospital

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A study was designed to assess the impact of the VITEK 2 automated system and the Advanced Expert System (AES) on the clinical laboratory of a typical university-based hospital. A total of 259 consecutive, nonduplicate isolates of *Enterobacteriaceae* members, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were collected and tested by the VITEK 2 system for identification and antimicrobial susceptibility testing, and the results were analyzed by the AES. The results were also analyzed by a human expert and compared to the AES analyses. Among the 259 isolates included in this study, 245 (94.6%) were definitively identified by VITEK 2, requiring little input from laboratory staff. For 194 (74.9%) isolates, no inconsistencies between the identification of the strain and the antimicrobial susceptibility determined by VITEK 2 were detected by the AES. Thus, no input from laboratory staff was required for these strains. The AES suggested one or more corrections to results obtained with 65 strains to remove inconsistencies. The human expert thought that most of these corrections were appropriate and that some resulted from a failure of the VITEK 2 system to detect certain forms of resistance. Antimicrobial phenotypes assigned to the strains by the AES for β-lactams, aminoglycosides, quinolones, macrolides, tetracyclines, and glycopeptides were similar to those assigned by the human expert for 95.7 to 100% of strains. These results indicate that the VITEK 2 system and AES can provide accurate information in tests for most of the clinical isolates examined and remove the need for human analysis of results for many. Certain problems were identified in the study that should be remediable with further work on the software supporting the AES.

The VITEK 2 system is a new system that automatically performs rapid identification and antimicrobial susceptibility testing on a manually prepared inoculum (1). The Advanced Expert System (AES) is designed to analyze results generated by the VITEK 2 system for biologic validity and then provide comments on the results. One important function of the AES is to look for inconsistencies between the identification of the organism and the antimicrobial susceptibility of the isolate. Another important function is to ascertain the antimicrobial phenotype of the isolate based on results of susceptibility tests. A third function is to deduce the susceptibility of the organism to drugs not tested based on its susceptibility to the antibiotics actually tested.

In a previous study, the ability of the AES to correctly ascertain the β-lactam phenotype of isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* was determined using a panel of 196 strains collected worldwide which had been characterized by biochemical and molecular techniques for their β-lactamase content (6). The results of that study showed that the AES correctly identified the β-lactam phenotype of 183 (93.4%) of these isolates despite the inclusion of many rare phenotypes in the isolate panel. The study, however, did not assess the potential impact of the VITEK 2 system and AES on the workflow of a clinical laboratory in a typical hospital since the isolates examined had been selected for their known and sometimes quite rare resistance mechanisms. Furthermore, only the accuracy of the AES for determining the β-lactam phenotypes was determined in the study.

Therefore, a study was designed to ascertain the potential impact of the VITEK 2 system and AES on a typical clinical laboratory located in a university-based hospital. For this study, consecutive nonduplicate isolates of gram-negative rods and gram-positive cocci isolated and identified from specimens by the laboratory were collected and tested by the VITEK 2 system for identification and antibiotic susceptibility. The AES was used to analyze and interpret results generated by the VITEK 2 system. The results were also analyzed by a human expert (CCS) and compared to those of the AES.

MATERIALS AND METHODS

Study design. Consecutive, nonduplicate isolates were collected from St. Joseph Hospital, Omaha, Neb., a 400-bed tertiary-care hospital. The microbiology laboratory services not only the hospital but also a large outreach program that includes physician clinics, large group practices, and approximately 20 nursing homes. During 1999, the laboratory processed approximately 88,780 specimens, performing 5,134 blood cultures, 11,215 urine cultures, 3,434 throat cultures, 4,390 wound cultures, 2,062 respiratory system cultures, 1,074 stool cultures, and 7,562 susceptibility tests.

The isolates collected included a total of 300 gram-negative rods and gram-positive cocci. Among the isolates collected, only those that would routinely be

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identified to the species level by the clinical laboratory were included in the study. This inclusion criterion was based on the fact that the information generated by the AES is of greatest utility for isolates identified to the species level. Thus, from the 300 consecutive nonduplicate isolates, 259 were selected for the study; these included 170 *Enterobacteriaceae* members, 41 *P. aeruginosa* isolates, and 48 *Staphylococcus aureus* isolates.

Isolates were inoculated into the VITEK 2 system for identification and susceptibility testing. In the few instances in which the VITEK 2 was unable to provide a definitive identification, the species identification as determined in routine tests by the clinical laboratory was manually entered so that the AES could analyze the results.

Identification and susceptibility tests. All identifications and susceptibilities were determined using the VITEK 2 system, as recommended by the manufacturer. VITEK 2 ID-GNB and ID-GPC cards were used for identification of gram-negative and gram-positive bacteria, respectively. The VITEK 2 susceptibility cards used in this study were standard European configurations and contained the following antibiotics and concentration ranges: (i) AST-N009 for *Enterobacteriaceae*—ampicillin 2 to 32 μg/ml, amoxicillin-clavulanate (2:1 ratio) 2 and 1 to 32 and 16 μg/ml, cephalothin 2 to 64 μg/ml, cefoxitin 4 to 64 μg/ml, cefotaxime 1 to 64 μg/ml, ceftazidime 1 to 64 μg/ml, ticarcillin 8 to 128 μg/ml, ticarcillin-clavulanate (clavulanate at 2 μg/ml with ticarcillin a twofold dilution) 8 and 2 to 128 and 2 μg/ml, piperacillin-tazobactam (tazobactam at 4 μg/ml with piperacillin a twofold dilution) 4 and 4 to 128 and 4 μg/ml, imipenem 0.5 to 16 μg/ml, amikacin 2 to 64 μg/ml, gentamicin 1 to 16 μg/ml, netilmicin 1 to 32 μg/ml, tobramycin 1 to 16 μg/ml, nalidixic acid 2 to 32 μg/ml, ciprofloxacin 0.25 to 4 μg/ml, norfloxacin 0.5 to 16 μg/ml, ofloxacin 0.25 to 8 μg/ml, nitrofurantoin 16 to 512 μg/ml, and trimethoprim-sulfamethoxazole 20 to 320 μg/ml; (ii) AST-N008 for *P. aeruginosa*—cefepime 1 to 64 μg/ml, ceftazidime 1 to 64 μg/ml, piperacillin 4 to 128 μg/ml, piperacillin-tazobactam (tazobactam at 4 μg/ml with piperacillin a twofold dilution) 4 and 4 to 128 and 4 μg/ml, ticarcillin 8 to 128 μg/ml, ticarcillin-clavulanate (clavulanate at 2 μg/ml with ticarcillin a twofold dilution) 8 and 2 to 128 and 2 μg/ml, imipenem 0.5 to 16 μg/ml, meropenem 0.25 to 16 μg/ml, aztreonam 1 to 64 μg/ml, amikacin 2-64 μg/ml, gentamicin 1 to 16 μg/ml, netilmicin 1 to 32 μg/ml, tobramycin 1 to 16 μg/ml, isepamycin 1 to 64 μg/ml, colistin 0.5 to 16 μg/ml, pefloxacin 0.25 to 16 μg/ml, fosfomycin 4 to 128 μg/ml, and trimethoprim-sulfamethoxazole 20 to 320 μg/ml; (iii) AST-P515 for *Staphylococcus* spp.—benzylpenicillin 0.03 to 0.5 μg/ml, clindamycin 0.25 to 8 μg/ml, erythromycin 0.25 to 8 μg/ml, fosfomycin 8 to 128 μg/ml, fusidic acid 0.5 to 32 μg/ml, gentamicin 0.5 to 16 μg/ml, kanamycin 8 to 32 μg/ml, lincomycin 2 to 16 μg/ml, minocycline 4 to 16 μg/ml, nitrofurantoin 16 to 512 μg/ml, norfloxacin 0.25 to 16 μg/ml, ofloxacin 0.5 to 8 μg/ml, oxacillin (breakpoint test) S to R, oxacillin MIC 0.5 to 8 μg/ml, pristinamycin 0.5 to 8 μg/ml, rifampin 0.5 to 32 μg/ml, teicoplanin 0.5 to 32 μg/ml, tetracycline 1 to 16 μg/ml, tobramycin 4 to 16 μg/ml, trimethoprim-sulfamethoxazole 10 to 320 μg/ml, and vancomycin 1 to 32 μg/ml. Quality control was performed daily during testing, using *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for gram-negative bacteria and *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 for gram-positive bacteria. β-Lactamase determinations were made by the chromogenic cephalosporin assay (4).

The AES version evaluated in this study was X01.00P, in conjunction with VITEK 2 software version T01.01.0038. The interpretation standard "NCCLS" was used with the interpretation guideline "Natural Resistance" and the parameter set "US hospitals." Results obtained with any antibiotic for which no NCCLS interpretive criteria existed were not evaluated in this study (2).

AES testing. In the biological validation phase, the AES examines the antimicrobial susceptibility data and determines if the MICs obtained are consistent with the species identification of the organism. If a single error is found, the AES recommends either a change in the identification that will make the outlying MIC consistent or a numeric change in the MIC that will make it consistent with the identification. These recommendations are considered biological corrections because AES presumes that (i) an error has occurred in the data generated by the VITEK 2, (ii) results were atypical due to the strain, (iii) a "falsely" negative result has occurred (e.g. noninduced β-lactamase), or (iv) an incorrect result was entered manually by a technologist. A biological correction is recommended by the AES if it detects only a single MIC inconsistency. The AES will recommend retesting the isolate if more than one biological correction would be needed to bring the susceptibility in line with the identification or to match phenotypes (see below).

During the biological validation phase, the AES may also recommend biological corrections based on the phenotype of the organism (see below for explanation of phenotypes). For example, any β-lactamase-positive *S. aureus* strain is resistant to penicillin. If the penicillin MIC generated by the VITEK 2 system is not in the resistant range for a β-lactamase-positive strain, the AES makes a biological correction to the MIC. Similarly, any discrepancies between the oxacillin screen and the oxacillin MIC are corrected by the AES with a biological correction.

A second type of correction suggested by the AES is a therapeutic correction. Unlike the biological correction, no bacteriological error is presumed in the data with a therapeutic correction; thus, the species name and numeric value of the MIC remain unchanged. However, the interpretation whether the MIC indicates susceptibility (S), intermediate susceptibility (I), or resistance (R) is altered in a therapeutic correction. For example, MICs of trimethoprim-sulfamethoxazole may be in the susceptible range for a few strains of *P. aeruginosa* although it is well established that this species is uniformly resistant to the drug. With such a strain, the AES will make a therapeutic correction to the interpretation of the MIC, changing the (S) to an (R). The numeric value of the MIC is not changed since it is not likely to be in error, i.e., since repeat tests would generate the same MIC. Since therapeutic corrections do not imply errors in the data, the AES may suggest multiple therapeutic corrections for a single strain.

Once the AES has analyzed results for consistency between identification and MICs, it produces a statement that (i) results are consistent with identification, (ii) results are not fully consistent and a single biological correction and/or one or more therapeutic corrections are suggested to remove the inconsistency, or (iii) results are not fully consistent and the isolate should be retested. This third option is suggested when there appears to be more than one biological error or when several possibilities for correction exist. Strains for which any biologic or therapeutic corrections have been suggested require a member of the laboratory staff to determine whether the corrections suggested by the AES should be accepted. Once this decision has been made, MIC results are finalized by the AES.

During biological validation, the AES examines the MIC data for each class of drug tested to determine a phenotype for the strain. The phenotype in this context refers to the expression of specific mechanisms of susceptibility or resistance to a given drug class within a particular species. Thus, with any species and any particular class of drug, there is usually a single wild-type phenotype and one or more resistance phenotypes. The wild-type phenotype is the drug class susceptibility for the species in the absence of any mutations in chromosomal genes or acquisition of extrachromosomal genes for resistance to the drug class. For β-lactam drugs, most resistance phenotypes reflect the type of β-lactamase(s) likely to be produced by the strain, the permeability of the strain to the drugs, and/or the presence of an altered target (e.g., methicillin-resistant *S. aureus*). For aminoglycosides, resistance phenotypes often reflect the type of inactivating enzyme(s) produced. For macrolides, an altered target is a common resistance phenotype, while for tetracyclines, efflux pumps are common resistance phenotypes.

For each class of drug tested by the VITEK 2 system, the AES attempts to determine a phenotype for the strain. This is done by comparing the measured MICs of the drugs within a class to a range of MICs in the AES database for strains possessing documented phenotypes (6). If the MICs obtained with the strain fall within the range expected for a specific phenotype, the strain is assigned that phenotype. However, if the MICs obtained with the strain fall within the range expected for more than one phenotype, the AES lists each of those matching phenotypes without identifying which one may be most likely.

With certain resistance phenotypes, the AES may suggest therapeutic corrections. For example, it is well established that MICs for certain β-lactam antibiotics in tests with some *Enterobacteriaceae* which produce extended-spectrum β-lactamases (ESBLs) are not in the resistant range despite clear demonstration of clinical failures of the drugs if used in therapy. If the AES determines that a strain of *E. coli* or *Klebsiella* has an ESBL phenotype, it will suggest a therapeutic correction (change S to R) for MICs of any relevant β-lactam antibiotic as recommended by the NCCLS (2).

The AES also deduces susceptibility to drugs not tested based on the susceptibility of the strain to the antibiotics actually tested by VITEK 2. The drugs for which susceptibility can be deduced by the AES are dependent on the drugs actually tested and the antimicrobial phenotype of the strain. Furthermore, the user of the AES may select which drugs he or she wishes to have deduced among those available. For this study, only susceptibility to cefaclor was deduced for the gram-negative rods tested while susceptibility to certain macrolides and β-lactams was deduced for staphylococci. The basis for deduced susceptibility is the antimicrobial phenotype assigned to the strain by the AES. If, for example, the AES has determined that the β-lactam phenotype of a strain is acquired penicillinase, then among the drugs selected for deduction by the user, the AES will deduce susceptibility if all strains with this phenotype are known to be susceptible to the drug or resistance if all strains with this phenotype are known to be resistant to the drug. If some strains with this phenotype are susceptible and some are resistant to the drug in question, the AES will show in the expanded

TABLE 1. Discrepancies in identification of study isolates

Strain	Identification provided by:	
	Clinical laboratory	VITEK 2[a]
476	*K. pneumoniae*	*K. pneumoniae* subsp. *ozaenae* or *Serratia rubidaea*
535	*K. pneumoniae*	*K. pneumoniae* or *E. aerogenes*
488	*K. pneumoniae*	*K. pneumoniae* or *E. aerogenes*
465	*K. pneumoniae*	*K. oxytoca* or *K. pneumoniae*
434	*K. pneumoniae*	*K. pneumoniae* or *K. oxytoca*
401	*K. pneumoniae*	*K. pneumoniae* or *K. pneumoniae* subsp. *ozaenae*
608	*K. pneumoniae*	Unidentified organism
569	*K. pneumoniae*	Unidentified organism
490	*E. cloacae*	Unidentified organism
486	*E. cloacae*	Card terminated—no organism suspension detected
546	*E. coli*	*C. freundii* or *E. cloacae*[b]
466	*E. coli*	Unidentified organism
409	*P. aeruginosa*	*P. aeruginosa* or *Burkholderia pseudomallei*[c]
483	*P. aeruginosa*	*P. aeruginosa* or various nonfermentative gram-negative bacilli[c]

[a] Instances of two possible identifications represent a confidence level reflecting low discrimination.
[b] Susceptible to all β-lactam drugs including ampicillin.
[c] Gave warning of highly pathogenic organism.

report that S, I, and R all remain possible, and the drug will not be deduced on the VITEK 2 report.

Assessment of the AES. Once the AES had analyzed results, a hard copy of the VITEK 2 laboratory report along with AES comments was printed and given to a human expert (CCS) for evaluation. The validity of any biological correction or therapeutic correction made during the biological validation phase was assessed. Phenotype assignment was evaluated by the human expert, as was any therapeutic correction made by the AES based on phenotype. Finally, susceptibility to drugs not tested and deduced by the AES was evaluated.

RESULTS

Identification. Among the 259 isolates, a definitive species was provided for 245 (94.6%) by the VITEK 2 system. This included 36 strains within the genus *Klebsiella* for which an indole test had to be performed by a technologist before a final species could be designated. Otherwise, no input from laboratory staff was required for species determination. There were 14 strains for which the VITEK 2 could not provide a definitive identification. These are listed in Table 1. Among the *Enterobacteriaceae*, the VITEK 2 had low discrimination on six strains identified by the clinical laboratory as *Klebsiella pneumoniae*, although *K. pneumoniae* was one of the two possible species listed for each of the strains. One strain with low discrimination by VITEK 2 was identified by the clinical laboratory as *E. coli*. Although VITEK 2 listed *Citrobacter freundii* or *Enterobacter cloacae* as possible species, the strain was susceptible to all β-lactam drugs tested including ampicillin, suggesting that *E. coli* was most likely to be correct. Five additional strains were not identifiable by the VITEK 2 system. Two of these were identified as *K. pneumoniae* by the clinical laboratory, two were identified as *E. cloacae*, and one was identified as *E. coli* (Table 1). Two strains identified as *P. aeruginosa* by the clinical laboratory had low discrimination by VITEK 2 (Table 1). Each was listed as possibly *P. aeruginosa* or a highly pathogenic organism by VITEK 2.

Among the 259 strains tested, the final number of strains of each species was 78 *E. coli*, 29 *K. pneumoniae* (including *K. pneumoniae* subsp. *ozaenae*), 7 *Klebsiella oxytoca*, 15 *E. cloacae*, 3 *Enterobacter aerogenes*, 5 *Citrobacter koseri*, 1 *Citrobacter amalonaticus*, 4 *Citrobacter freundii*, 13 *Proteus mirabilis*, 3 *Morganella morganii*, 4 *Providencia stuartii*, 8 *Serratia marcescens*, 41 *P. aeruginosa*, and 48 *S. aureus*.

Biological validation of results. Among the 259 strains tested, the AES found the results with 194 (74.9%) to be fully consistent, suggesting no biological corrections or therapeutic corrections (this excludes therapeutic corrections based on phenotype, which are considered below). This included 122 *Enterobacteriaceae*, 33 *P. aeruginosa*, and 39 *S. aureus*. The human expert agreed with the AES on 193 of these strains. The sole exception was *Providencia stuartii* 613, for which the MICs of ciprofloxacin, ofloxacin, norfloxacin, and nalidixic acid were 4, 8, 4, and 32 μg/ml, respectively. The human expert would have made a biological correction to the norfloxacin MIC to 4 μg/ml.

There were a total of 65 strains for which the AES suggested one or more corrections (Table 2). These would have required a staff member to review the suggested corrections and approve or dismiss each before a final readout on the strain was available. The single strain of *P. mirabilis* that the AES suggested retesting appeared to have errors in the MICs of imipenem and cefoxitin, indicating resistance. Failure of the VITEK 2 system to detect resistance to ampicillin and/or amoxicillin-clavulanate among strains of *C. freundii, E. cloacae*, or *E. aerogenes* was responsible for the AES suggesting a biological correction to the MICs of the drugs and/or correction in the identification (Table 2). The inability of the VITEK 2 to detect resistance to ampicillin and/or ticarcillin among strains of *K. pneumoniae* and *K. oxytoca* was responsible for most of the therapeutic corrections suggested by the AES with these organisms. False resistance to imipenem indicated by VITEK 2 was responsible for the biological corrections suggested by the AES for *P. mirabilis* (Table 2). Among the *P. aeruginosa* isolates, therapeutic corrections for trimethoprim-sulfamethoxazole were suggested by the AES for six strains for which the MICs were in the susceptible range. For *S. aureus*, discrepancies between the oxacillin MIC and oxacillin screen were responsible for most of the corrections suggested by the AES with this species (Table 2).

The human expert did not fully agree with the AES on 5 of the 65 strains (Table 2). These included a strain of *C. freundii*

TABLE 2. Outcome of the biological validation phase of AES[a]

Species	No. tested	No. fully consistent	No. with only TC to susceptibility result	Inconsistencies			
				No. retested	No. of BC to susceptibility result[b] or correct ID or retested	No. with correct ID or retested	No. of BC to susceptibility result[b] or retested
C. koseri	5	5					
C. amalonaticus	1	1					
C. freundii	4	1			2[c]	1	
E. aerogenes	3	2			1		
E. cloacae	15	9	1 (AM)		3	2	
E. coli	78	78					
K. oxytoca	7	1	6 (TIC [4], AM+TIC [2])				
K. pneumoniae	29	10	18 (TIC [8], AM+TIC [10])		1[c]		
M. morganii	3	3					
P. stuartii	4	0	4 (GM+NET [1], GM+NET+TM [3])				
P. mirabilis	13	6		1			6
S. marcescens	8	6	2 (AM+AMC)				
P. aeruginosa	41	33	6 (SXT)				2[c]
S. aureus	48	39	1 (PEN)		3		5[c]
Total	259	194 (74.9%)	38	1	10	3	13

[a] Abbreviations: ID, identification; BC, biological correction; TC, therapeutic correction; AM, ampicillin; TIC, ticarcillin; AMC, amoxicillin-clavulanate; SXT, trimethoprim-sulfamethoxazole; PEN, penicillin; GM, gentamicin; NET, netilmicin; TM, tobramycin.
[b] May also have therapeutic correction to susceptibility result.
[c] Includes one or more strains for which the human expert disagreed with AES.

for which the AES suggested a biological correction and therapeutic correction to the ceftazidime MIC (change 4 μg/ml S to 16 μg/ml 1) due to the high-level cephalosporinase phenotype of the strain or suggested a change of the identification to *E. cancerogenus*. The human expert felt that there were no inconsistencies in the data and no corrections were needed. The AES suggested either (i) changing the identification of a strain of *K. pneumoniae* with an acquired penicillinase phenotype to *K. ornithinolytica* and making a therapeutic correction to ticarcillin or (ii) keeping the identification, changing the cephalothin MIC from 2 μg/ml S to 8 μg/ml S, and making a therapeutic correction to ticarcillin. The human expert felt that there were no inconsistencies in the data and no corrections were needed. For two strains of *P. aeruginosa* with a high-level resistance phenotype, the AES suggested a biological correction to the ceftazidime MIC (4 μg/ml S to 8 μg/ml R). The human expert felt that both a biological correction and therapeutic correction should be made to the ceftazidime MIC (4 μg/ml S to 8 μg/ml R). One strain of *S. aureus* appeared susceptible to penicillin and oxacillin on the basis of MIC but had an oxacillin screen indicating resistance. The AES suggested changing the screen to indicate susceptibility or to retest as an unusual resistance was observed. The human expert felt that the strain should be retested.

Phenotypes. The AES was able to identify a phenotype for β-lactams, aminoglycosides, and quinolones for 207 of the 211 gram-negative rods tested (Table 3). The four strains for which a phenotype could not be identified had so many inconsistencies in the data that the AES suggested retesting or a making change in the identification. These included *C. freundii* 456, which appeared susceptible to ampicillin, amoxicillin-clavulanate, cephalothin, and cefoxitin; *E. cloacae* 490 and 457, which appeared susceptible to ampicillin and amoxicillin-clavulanate; and *P. mirabilis* 481, which appeared resistant to cefoxitin and imipenem.

Among the 207 strains for which a phenotype was assigned by the AES, the human expert agreed with the AES for 198 (95.7%) of the β-lactam phenotypes, 202 (97.6%) of the aminoglycoside phenotypes, and 204 (98.5%) of the quinolone phenotypes (Table 3). The strains for which there was a disagreement in the phenotype are listed in Table 4. Most of the disagreements were minor and included instances of the AES invoking a resistance phenotype for quinolones or aminoglycosides based on MICs being elevated above those seen with the wild type but not in the frankly resistant category. With strains of *P. aeruginosa* that were resistant to ticarcillin and ticarcillin-clavulanate but susceptible to piperacillin, the AES assigned a phenotype of acquired penicillinase (Table 4). The human expert assigned a wild-type phenotype to these strains based on previous work with this species, indicating that most strains of this species in the United States with acquired penicillinase are resistant to all antipseudomonal penicillins and wild-type strains may be resistant to ticarcillin and ticarcillin-clavulanate (5, 6).

With several strains, there was agreement between the AES and the human expert as to the antimicrobial phenotype; however, the two disagreed about what therapeutic corrections would be appropriate based on the assigned phenotype (Table 5). For three strains with a resistant β-lactam phenotype, the human expert felt that therapeutic corrections should be made to several drugs (Table 5). With the AES software used for this study, no therapeutic corrections were made for any quinolone phenotype; i.e., if a strain appeared resistant to all but one fluoroquinolone tested, the AES did not make a therapeutic correction (change S to R) to the single drug to which the strain appeared susceptible. This created a problem with norfloxacin, whose MICs tended to be lower for some species than were those of ciprofloxacin. Thus, several strains appeared susceptible to norfloxacin but resistant to ciprofloxacin and other fluoroquinolones tested. The human expert felt that this

TABLE 3. Agreement between AES and human expert on phenotypes for the gram-negative rods

Species	No. of strains phenotyped	β-lactams			Aminoglycosides			Quinolones		
		Correct[a]	Correct 1+[b]	Disagree[c]	Correct	Correct 1+	Disagree	Correct	Correct 1+	Disagree
C. koseri	5		4	1		5		5		
C. amalonaticus	1	1			1			1		
C. freundii	3	1	1	1		3		2		1
M. morganii	3	2		1		3		1		2
K. oxytoca	7	6	1			7		7		
K. pneumoniae	29	29			2	27		26	3	
E. aerogenes	3	3				3		3		
E. cloacae	13	12		1	1	12		12	1	
P. mirabilis	12	12			1	11		7	5	
S. marcescens	8	6	1	1	1	7		8		
P. stuartii	4	4			4			4		
E. coli	78	75	2	1		78		72	6	
P. aeruginosa	41	34	4	3	30	6	5	41		
Total	207	185	13	9	40	162	5	189	15	3

[a] AES listed a single phenotype and the human expert agreed.
[b] AES listed more than one phenotype and the human expert felt that the correct phenotype was among those listed by the AES and that other alternatives listed by the AES were reasonable possibilities.
[c] Phenotype(s) listed by AES did not include the phenotypes selected by the human expert, or AES listed some phenotypes that the human expert thought were unlikely.

problem could be resolved by therapeutic corrections to norfloxacin (Table 5).

For tests with the 48 strains of *S. aureus*, the AES and human expert agreed on phenotypes assigned to all of the strains for the aminoglycosides, macrolides, quinolones, glycopeptides, and tetracyclines. There was a single disagreement on phenotypes for the β-lactams. This was a β-lactamase-negative strain for which the AES assigned a borderline phenotype and the human expert assigned a wild-type phenotype.

Deduced drugs. The use of phenotypes to deduce the susceptibility to drugs not tested led to a problem with cefaclor and *E. coli*. The MIC of cephalothin for certain strains of *E. coli* fell in the 1 range, while those of all other β-lactams tested were in the S range. This pattern was designated the wild-type phenotype by the AES. Accordingly, all wild-type *E. coli* strains are considered susceptible to cefaclor by deduction. This created a problem of strains testing I to cephalothin but having S deduced for cefaclor—a clear violation of current NCCLS recommendations that susceptibility to one indicates susceptibility to both and resistance to one indicates resistance to both (2).

DISCUSSION

The results of this study indicated that the VITEK 2 system and AES could be used on the majority of clinical isolates encountered in a typical university-based laboratory for iden-

TABLE 4. Disagreement between AES and human expert on phenotypes assigned to gram-negative rods

Drug group	Strain	Phenotype provided by:	
		Human expert[d]	AES[d]
β-Lactams	*C. freundii* 581	High-level cephalosporinase or ESBL[a]	High-level cephalosporinase
β-Lactams	*M. morganii* 511	High-level cephalosporinase or ESBL[a]	High-level cephalosporinase
β-Lactams	*C. koseri* 534	Wild type[b]	ESBL/wild type/acquired penicillinase
β-lactams	*E. cloacae* 588	ESBL or high-level cephalosporinase	Wild type
β-Lactams	*S. marcescens* 610	ESBL or high-level cephalosporinase[a]	High-level cephalosporinase
β-Lactams	*E. coli* 438	Permeability	Wild type
β-Lactams	*P. aeruginosa* 492	Wild type	Acquired penicillinase
β-Lactams	*P. aeruginosa* 520	Wild type	Acquired penicillinase
β-Lactams	*P. aeruginosa* 599	Wild type	Acquired penicillinase
Quinolones	*C. freundii* 581	Partial resistance	Resistant[c]
Quinolones	*M. morganii* 511	Wild type	Partial resistance[c]
Quinolones	*M. morganii* 538	Wild type[b]	Wild type/resistant quinolones-1/partial resistance[c]
Aminoglycoside[d]	*P. aeruginosa* 409	Wild type	Wild type/Gen and Net resistant/Gen resistant[c]
Aminoglycoside	*P. aeruginosa* 414	Wild type	Wild type/Gen and Net resistant/Gen resistant[c]
Aminoglycoside	*P. aeruginosa* 506	Wild type	Wild type/Gen and Net resistant/Gen resistant[c]
Aminoglycoside	*P. aeruginosa* 492	Net resistant	Gen and Net resistant/Gen Net, and Ami resistant[c]
Aminoglycoside	*P. aeruginosa* 544	Wild type	Wild type/Gen and Net resistant/Gen Net and Ami resistant[c]

[a] Minor disagreement, but second phenotype equally possible not listed by AES.
[b] Minor disagreement, but additional phenotypes listed by AES unlikely.
[c] MICs elevated above wild type but not all in resistant range.
[d] Abbreviations: Gen, gentamicin; Net, netilmicin; Ami, amikacin.

TABLE 5. Additional therapeutic corrections suggested by the human expert based on phenotype assigned by both the AES and human expert

Strain	Phenotype	Therapeutic correction suggested by human expert[a]
M. morganii 511	High-level cephalosporinase	Change cefoxitin 8S to 8R
E. cloacae 577	High-level cephalosporinase	Change piperacillin-tazobactam 32I to 32R
P. aeruginosa 544	High-level resistance	Change ceftazidime 16I to 16R and aztreonam 16I to 16R
E. cloacae 586	Resistant	Change norfloxacin 8I to 8R
E. cloacae 588	Resistant	Change norfloxacin 8I to 8R
E. cloacae 577	Resistant	Change norfloxacin 8I to 8R
S. marcescens 442	Resistant	Change norfloxacin 2S to 2R
P. stuartii 468	Resistant	Change norfloxacin 2S to 2I
P. stuartii 449	Resistant	Change norfloxacin 8I to 8R

[a] Correction indicated by value of MIC (in micrograms per milliliter) and interpretation of the MIC is S, I, or R.

tification and antimicrobial susceptibility testing with little or no input from laboratory staff concerning the interpretation of results. Of the 259 isolates included in this study, 94.6% were definitively identified by VITEK 2 and 74.6% had no inconsistencies between the identification of the strain and the antimicrobial susceptibility results. Of the 65 strains for which a correction was identified by AES, 58.5% required only a therapeutic correction to the susceptibility results. Most of these were due to the failure of the VITEK 2 system to detect β-lactam resistance in organisms possessing an intrinsic β-lactamase—a failure common to test systems that are rapid or involve small inocula (7). The AES has been designed to look for such problems in the data and correct them when found. However, if false susceptibility due to this problem occurs with two or more β-lactam drugs in tests with the same strain, the AES must recommend retesting the isolate. This occurred with a number of the strains of *Enterobacter* spp. and *C. freundii* listed Table 2 as possibly needing retesting. Also of concern was the false resistance to imipenem among 7 of the 13 isolates of *P. mirabilis* encountered in this study. Although the AES indicated to the user that this result was probably incorrect, this problem with the VITEK 2 system should ultimately be resolved in the algorithm used to determine susceptibility results. Since imipenem resistance, although rare among *Enterobacteriaceae*, can occur in *P. mirabilis* (3), it is imperative to be able to ascertain when resistance is real and when it is a problem with the test system.

There was very good agreement between the human expert and the AES in recognizing inconsistencies in this study. For only 5 (7.7%) of the 65 strains identified by the AES as needing corrections to the data did the human expert disagree with the AES about whether an inconsistency existed or how to correct the inconsistency. The major limitation of the AES noted in the biological validation phase of data analysis was its inability to recognize a single pattern of inconsistency and correct it. For example, false susceptibility to ampicillin, amoxicillin-clavulanate, cephalothin, and/or cefoxitin occurred with a few strains of *Enterobacter* spp. or *C. freundii*. To the AES, this was more than one inconsistency and required repeat testing or a change in identification of the organism with or without biologic and therapeutic corrections. To the human expert, this multiple drug inconsistency was due to a single problem—failure to detect intrinsic resistance due to low-level expression of a chromosomal β-lactamase. Repeat testing of these strains generated the same inconsistent data and the same AES analysis (data not shown) because the problem was intrinsic to the VITEK 2 system and could not be resolved by repeat testing. It should be noted that the design of the AES prevents it from making corrections if two or more inconsistencies are identified. Thus, it cannot recognize single-source problems that lead to multiple inconsistencies.

Agreement between the human expert and the AES was also very good concerning the identification of antimicrobial phenotypes. Overall agreement across the different drug groups varied from 95.7 to 100%. This is similar to results obtained in an earlier study that reported 97.3% agreement between a human expert and an expert system linked to a rapid antibiotic susceptibility test system (8). The major limitation noted for the AES was its inability to rank in order the various phenotypes among the possible phenotypes when more than one matched the MIC distribution. At times, as many as four different phenotypes would be listed by the AES with no indication of which one or possibly two were the most likely. In certain instances, the AES listed the wild type as well as one or two different resistance phenotypes as possibilities. Clearly, there are instances where this can occur. However, it would be more helpful to the user if in these instances the AES gave a message that a specific phenotype could not be ascertained rather than listing multiple phenotypes.

The ability of the AES to deduce drug susceptibility was not thoroughly analyzed in this study since too few drugs were involved. However, inconsistencies between the NCCLS rules for interpretation of susceptibility to cephalothin and cefaclor appeared to be violated when the AES deduced susceptibility to cefaclor from results obtained with cephalothin. Once this problem was identified in the study, it was corrected, so that the most recent version of the AES allows the user to base deductions strictly on NCCLS recommendations.

For all laboratories, regardless of size and type of staffing, the availability of the VITEK 2 system and AES could have a very positive impact on both the work flow and the quality of the information leaving the laboratory. However, the precise impact of the VITEK 2 system and AES will vary depending on the laboratory involved. For larger, more complex laboratories with a human expert on staff, the VITEK 2 and AES could free up valuable time of the expert. It would no longer be necessary for the expert to spend time identifying minor inconsistencies, since once they were identified by the AES, the expert could readily resolve them by using the choices provided by the AES. In fact, once the expert becomes familiar with the corrections usually suggested by the AES for certain organism-drug combinations, the system can be set to automatically correct the

inconsistency, eliminating the need for external expert input in selected situations. Thus, more of the human expert's time could be spent dealing with the problematic issues identified by the AES, i.e., organisms with multiple inconsistencies. Identification of antimicrobial phenotypes by the AES would also be most useful for this type of laboratory. In instances where more than one phenotype is listed as possible by the AES, the human expert can select the single most likely phenotype to include in the final report sent out of the laboratory. Also, additional therapeutic comments based on the most likely phenotype may be custom designed by the human expert for inclusion in the final report to aid physician selection of therapy.

For smaller, less complex laboratories without a human expert, the availability of the VITEK 2 system and AES could significantly improve the quality of the information leaving the laboratory. Minor inconsistencies that could be overlooked or go unrecognized would be automatically identified by the AES, and corrections would be suggested before the final report was printed. Most laboratories have personnel with sufficient background to select the most appropriate correction for this type of data inconsistency, e.g. to make a therapeutic correction of I to R for ampicillin or ticarcillin for *K. pneumoniae*. However, in the setting of organisms with major inconsistencies, the laboratory staff may not be able to correctly choose between options provided by the AES, i.e., either make a correction in the identification or make a biological correction to an MIC. In this situation and instances where multiple biological inconsistencies are identified by the AES, repeat testing will probably be required. Unfortunately, if the same results are obtained on repeat testing, the recommendations of the AES will remain the same as before, leaving the laboratory personnel no further along in resolving the inconsistencies. The utility of the antimicrobial phenotypes identified by the AES will be more limited for the laboratory without a human expert. In instances where more than one phenotype is listed by the AES, it may not be possible for laboratory personnel to select the most likely phenotype. Thus, the final report sent out of the laboratory could be confusing to physicians if it contains a listing of possible phenotypes. It would be much more useful for this type of laboratory if the AES would rank the phenotypes listed in descending order of their likelihood or merely concede that a specific phenotype could not be ascertained.

In summary, the VITEK 2 system and AES can provide accurate information in tests with the majority of clinical isolates that are encountered in the typical university-based hospital. In addition, these new systems remove the need for human analysis of results for many isolates, freeing personnel for other activities and improving the overall quality of the information generated, especially in laboratories without a human expert. As with any microbiology system, the AES will require constant updating and modification to optimize its utility for the laboratory.

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