JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2004, p. 1542–1546 Vol. 42, No. 4
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.4.1542–1546.2004

Evaluation of the Automated Phoenix System for Potential Routine Use in the Clinical Microbiology Laboratory

J.-L. Donay,[1] D. Mathieu,[2] P. Fernandes,[1] C. Prégermain,[2] P. Bruel,[2] A. Wargnier,[1] I. Casin,[1] F. X. Weill,[1] P. H. Lagrange,[1] and J. L. Herrmann[1]*

Service de Microbiologie, Hôpital Saint Louis, Paris,[1] and Service de Microbiologie, Hôpital Paul Brousse, Villejuif,[2] France

Received 24 July 2003/Returned for modification 1 September 2003/Accepted 7 January 2004

A comparative study was designed to evaluate the identification (ID) and antimicrobial susceptibility testing (AST) performances of the BD Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems [BD], Pont de Claix, France). A total of 305 single clinical isolates were collected, and comparisons were made with routine manual methods in use in our microbiology laboratories. The percentages of correct IDs were 93.3, 89.4, 91.8, and 85.7% for enterobacteria, nonfermenting gram-negative bacilli, staphylococci, and streptococci-enterococci, respectively. The median ID time was 3 h, and the median time for AST was 10 h 30 min. AST results showed variable percentages of errors for the different antibiotics. None of the enterobacteria and 0.3% of *Pseudomonas aeruginosa* isolates showed a very major error (VME). Only one strain of *Staphylococcus aureus* showed a VME with oxacillin. We demonstrate here the efficiency of the Phoenix system, which can be used for the majority of strains encountered in a university-based laboratory, for ID and AST.

Automation in microbiology is not an easy task because microbiology is still a discipline that requires input from laboratory staff for the interpretation of results. This is specifically the case for bacterial identification (ID) and detection of antibiotic resistance, since microbiologists are often able to identify a bacterium and its resistance phenotype on the basis of only a few parameters. However, biochemical ID and susceptibility testing require 24 h to be readable. Several factors favor the use of automatic systems in the microbiology laboratory. Reproducibility, the ability to track results, the availability of results within one working day, and reduced amounts of contaminated waste represent the main reasons. An added advantage includes an automatic connection to the laboratory informatics software, allowing better management of technician staff, an easier validation for the microbiologist with the help of an expert system, and the opportunity for the clinician to obtain partial or complete results faster, thus improving patient management. Over the last 20 years, a variety of automated systems for ID and antimicrobial susceptibility testing (AST) have been developed (12, 13, 16, 17, 27, 33, 35, 36–41, 43). These automated technologies allowed for the very first time complete management of ID and AST, requiring technical staff to perform only one dilution from an agar plate culture. For example, the Vitek 2 (bioMérieux, Marcy l'Étoile, France) and the BD Phoenix (Becton Dickinson Diagnostic Systems [BD], Pont de Claix, France) are new systems that automatically perform ID and AST on a manually prepared inoculum (1, 3, 6, 9, 10, 14, 15, 18, 19, 22–25, 31, 42). Both designs possess an advanced expert system and have a potential impact on the workflow of a clinical laboratory for a typical hospital. Moreover, rapid ID and AST can have a significant impact on the management of infections, especially those caused by antibiotic-resistant bacteria (8, 36). The continuous challenge set by a high workload and limited resources and personnel encouraged us to evaluate the new Phoenix system for the potential routine use in two typical university-based microbiology laboratories. The data we present indicate that the Phoenix system could be used for ID and AST on the majority of clinical isolates encountered in our hospitals, and we assess the potential for this automated system to be used on a routine basis in a clinical laboratory.

* Corresponding author. Mailing address: Service de Microbiologie, Hôpital Saint Louis, 1 Ave. Claude Vellefaux, 75010 Paris, France. Phone: 33-1-42-49-93-48. Fax: 33-1-42-49-92-00. E-mail: jean-louis.herrmann@sls.ap-hop-paris.fr.

MATERIALS AND METHODS

Clinical strains. Between 5 and 20 October 2000, 305 single clinical isolates were studied by using the Phoenix system, and findings were compared to the results of the manual methods of ID and AST in use in our laboratories. These 305 strains included 130 *Enterobacteriaceae* strains, 57 non-lactose-fermenting gram-negative bacillus (NFGNB) strains, 76 *Staphylococcus* isolates, and 42 *Streptococcus* and *Enterococcus* isolates. The following species were included: *Escherichia coli* (69 isolates), *Klebsiella pneumoniae* (14 isolates), *Klebsiella oxytoca* (6 isolates), *Enterobacter cloacae* (12 isolates), *Enterobacter aerogenes* (7 isolates), *Citrobacter freundii* (6 isolates), *Proteus mirabilis* (7 isolates), *Proteus vulgaris* (1 isolate), *Proteus penneri* (1 isolate), *Serratia marcescens* (1 isolate), *Serratia liquefaciens* (1 isolate), *Hafnia alvei* (1 isolate), *Morganella morganii* (1 isolate), *Providencia stuartii* (1 isolate), *Salmonella enterica* (2 isolates), *Pseudomonas aeruginosa* (37 isolates), *Pseudomonas fluorescens* (2 isolates), *Pseudomonas putida* (1 isolate), *Stenotrophomonas maltophilia* (7 isolates), *Alcaligenes xylosoxidans* (2 isolates), *Acinetobacter baumanii* (5 isolates), *Acinetobacter lwoffi* (2 isolates), *Aeromonas hydrophila* (1 isolate), *Staphylococcus aureus* (42 isolates), *Staphylococcus epidermidis* (17 isolates), *Staphylococcus hominis* (7 isolates), *Staphylococcus haemolyticus* (7 isolates), *Staphylococcus warneri* (3 isolates), *Enterococcus faecalis* (23 isolates), *Enterococcus faecium* (5 isolates), *Enterococcus gallinarum* (1 isolate), *Streptococcus pneumoniae* (4 isolates), beta-hemolytic *Streptococcus* strains A, B, C, and G (7 isolates), and *Streptococcus mitis* (2 isolates) (the last three species were used only for ID).

Identification methods. (i) Phoenix identification. The method used for Phoenix identification was described previously (3, 10). Briefly, the Phoenix system uses one ID and AST combination panel with the ID substrates on one side and the antimicrobial drugs on the other side of the panel. Bacterial isolates were subcultured on Trypticase soy agar supplemented with 5% sheep blood agar (bioMérieux) to ensure the exclusion of contaminants. The Phoenix ID broth was inoculated with several bacterial colonies from a pure culture adjusted to 0.5 to

0.6 McFarland standard by using a CrystalSpec nephelometer (BD). After the transfer of 25 μl of the ID broth suspension to the Phoenix AST broth, the suspension was poured into the ID side of the Combo panel. Once inoculated, the panel was logged and loaded into the Phoenix Automate, in which kinetic measurements of colorimetric and fluorimetric signals were collected every 20 min. The suspension was always subcultured on Trypticase soy agar plus 5% sheep blood to ensure inoculum's purity, followed by incubation for 18 h at 37°C.

(ii) Comparative methods of identification. API systems (API-20E for enterobacteria, API-20NE for NFGNB, API-32 Staph for *Staphylococcus* spp., and API-32 Strept for *Streptococcus* and *Enterococcus* spp.; bioMérieux) were inoculated as recommended by the producer from the same agar pure culture.

For *Staphylococcus* spp. (especially *S. aureus*), coagulase production was used in addition to the Chapman medium (a hypersalted agar plate supplemented with mannitol [bioMérieux]). For *Enterococcus* spp., growth on bile-esculin, tellurite hydrolysis, and motility tests were performed.

AST. Twenty-nine antimicrobial drugs routinely tested in our laboratory were compared, including 16 beta-lactams, 4 aminoglycosides, 2 quinolones, 2 glycopeptides, and 5 other antimicrobial agents (see the tables). Breakpoints from le Comité de l'Antibiogramme de la Société Française de Microbiologie were used (4).

(i) Phoenix AST. The Phoenix AST method used was previously described (3, 10). Briefly, the Phoenix AST broth was supplemented by one drop of Phoenix AST indicator (oxidation-reduction indicator based on resazurin or Alamar blue). From the standardized ID suspension, 25 μl was transferred to the AST broth, resulting in a final inoculum density of ca. 5×10^5 CFU/ml. The broth was then poured into the AST side of the panel. As mentioned previously, the panel was loaded into the Phoenix apparatus. For each antibiotic, a minimum of eight concentrations (serial doubling dilutions) were tested. In addition, specific detection of staphylococcus penicillinase and extended-spectrum beta-lactamase (ESBL) for gram-negative bacteria was also performed on the AST side on the respective Combo panels.

(ii) Disk diffusion method. The AST method used in our laboratories is the disk diffusion method performed as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (4). For the assessment study phase, Mueller-Hinton agar plates with or without 5% sheep blood (Bio-Rad, Marnes la Coquette, France) were inoculated with the same AST broth dilution. The liquid suspension was then discarded, and disks with known concentrations of antibiotic were dispensed onto the agar plate. The measure of the antibiotic zone size was performed manually or with the Osiris system (Bio-Rad). Penicillinase detection for staphylococci was performed by the Cefinase disk method (AES, Combourg, France). The methicillin resistance of staphylococci was detected by a cefoxitin disk on the agar plate and the PCR-based *mecA* gene method as previously described (11). The ESBL detection was checked by a disk diffusion method set up in our laboratory. Briefly, cefotaxime and ceftazidime (broad-spectrum cephalosporins) disks were disposed at four different distances (2, 2.5, 3, and 3.5 cm) from a ticarcillin-clavulanic acid disk on a Mueller-Hinton agar plate with or without 100 μg of cloxacillin/ml (4, 29). The presence of a growth inhibition zone only between the cefotaxime or ceftazidime disk and the ticarcillin-clavulanic acid disk confirmed the synthesis of an ESBL by the bacterial isolate.

Data analysis and discrepancy resolution. For each antimicrobial drug tested by both methods, sensitive, intermediate, and resistant interpretative results were used. Category agreement (CA) corresponds to sensitive, intermediate, and resistant interpretative results matching between the two systems. Errors were classified as a very major error (VME) or a false-susceptible Phoenix result; a major error (ME) or a false-resistant Phoenix result; or a minor error (mE), i.e., with one system yielding an intermediate result and the other yielding a susceptible or resistant result (40). VME and ME findings were subjected to repeat testing by both the Phoenix and disk diffusion methods. Similarly, isolates with ID discrepancies, i.e., a different ID rendered by the Phoenix method compared to the API systems, were retested by using both Phoenix ID and the API ID methods. Discrepant isolates were then sent to the Reference Center Laboratory (Pasteur Institute, Paris, France) to ascertain species IDs by using additional biochemical tests and 16S RNA sequencing.

RESULTS

ID results. Of the 305 isolates tested during the present study phase of the Phoenix system, a concordant ID to the species level was obtained in 92.5% of cases. Enterobacteria and NFGNB showed a concordant result in 94.6 and 89.4% of cases, respectively (Table 1). Seven enterobacterial strains and six NFGNB showed a discordant Phoenix result with that of the respective reference method (Table 2). *E. coli* and *P. aeruginosa*, two major representatives of gram-negative bacilli encountered in the laboratory, showed a concordant result in 95.6 and 100% of cases, respectively. A 100% concordance was also observed for several enterobacteria and NFGNB (*K. oxytoca*, *Enterobacter aerogenes*, *C. freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *M. morganii*, *Stenotrophomonas maltophilia*, and *P. putida*). For *K. pneumoniae*, *Enterobacter cloacae*, *P. fluorescens*, *Alcaligenes xylosoxidans*, *A. baumanii*, and *A. lwoffi*, lower concordance rates of 92.8, 91.6, 50, 50, 40, and 50%, respectively, were found, but these organisms were represented by lower numbers of isolates.

TABLE 1. ID results for gram-positive and gram-negative bacteria

Organism group	No. of isolates tested	No. (%) of IDs	
		Concordant	Discordant
Enterobacteriaceae	130	123 (94.6)	7 (5.4)
NFGNB	57	51 (89.4)	6 (10.6)
Staphylococcus spp.	76	72 (91.8)	6 (7.7)
Enterococcus spp.	29	25 (86.2)	4 (13.8)
Streptococcus spp.	13	11 (84.6)	2 (15.4)
Total	305	282 (92.5)	23 (7.5)

Staphylococci, enterococci, and streptococci showed a concordant result in 91.8, 86.2, and 84.6% of cases, respectively (Table 1). A 100% concordance was observed for *S. aureus*, *S. hominis*, and beta-hemolytic streptococci. Single strains of *S. epidermidis*, *Staphylococcus haemolyticus*, *S. warneri*, and *Streptococcus mitis* showed a Phoenix ID discordant with that of the respective reference method (Table 2). Two strains of *E. faecalis* and one strain of *E. faecium* were misidentified by the Phoenix as *E. casseliflavus-gallinarum* (Table 2). One strain of *Streptococcus pneumoniae* was misidentified by the Phoenix as *Streptococcus mitis*, and one strain of *Streptococcus mitis* was

TABLE 2. Details of the misidentifications observed between Phoenix and the reference methods

Phoenix ID	*n*	NRC ID[a]
Shigella flexneri	2	*Escherichia coli*
Escherichia coli	1	*Salmonella* spp.
Klebsiella oxytoca	1	*Klebsiella pneumoniae*
Citrobacter brakii	1	*Enterobacter cloacae*
Salmonella enterica	1	*Escherichia coli*
Serratia marcescens	1	*Serratia liquefaciens*
Pseudomonas putida	1	*Pseudomonas fluorescens*
Pseudomonas oryzihabitans	1	*Alcaligenes xylosoxidans*
Moraxella spp.	3	*Acinetobacter* spp.
Acinetobacter baumannii	1	*Acinetobacter lwoffi*
Staphylococcus capitis	1	*Staphylococcus epidermidis*
Staphylococcus epidermidis	1	*Staphylococcus hominis*
Staphylococcus simulans	1	*Staphylococcus haemolyticus*
Staphylococcus caprae	1	*Staphylococcus warneri*
Staphylococcus aureus	1	*Staphylococcus hyicus*
Staphylococcus epidermidis	1	*Staphylococcus xylosus*
Enterococcus casseliflavus-gallinarum	2	*Enterococcus faecium*
Enterococcus casseliflavus-gallinarum	1	*Enterococcus faecalis*
Streptococcus anginosus	1	*Enterococcus faecalis*
Streptococcus mitis	1	*Streptococcus pneumoniae*
Streptococcus pneumoniae	1	*Streptococcus mitis*

[a] NRC, National Reference Center.

TABLE 3. Susceptibility tests: percentages of corresponding results between the Phoenix and agar diffusion methods

Antimicrobial agent[a]	% Susceptibility											
	E. coli (*n* = 55)				Other enterobacteria (*n* = 70)				*P. aeruginosa* (*n* = 32)			
	CA	mE	ME	VME	CA	mE	ME	VME	CA	mE	ME	VME
AMX	95.8	4.2	0	0	98.6	1.4	0	0				
AMC	81	19	0	0	95.7	2.9	1.4	0				
TIC									56.7	43.3	0	0
PIP	97.9	2.1	0	0	92.9	5.7	1.4	0	73.6	23.3	3.1	0
TZP	97.9	0	2.1	0	98.6	1.4	0	0	70.6	20	9.4	0
CEF	79	21	0	0	95.7	2.9	1.4	0				
CXM	97.9	2.1	0	0	100	0	0	0				
FOX	100	0	0	0	95.7	4.3	0	0				
CTX	97.9	2.1	0	0	95.7	4.3	0	0				
FEP									70	30	0	0
CAZ	100	0	0	0	100	0	0	0	70	23.2	3.4	3.4
IPM	100	0	0	0	100	0	0	0	100	0	0	0
ATM	100	0	0	0	100	0	0	0	36.7	63.3	0	0
GEN	100	0	0	0	100	0	0	0	73.3	16.7	10	0
TOB	100	0	0	0	98.6	1.4	0	0	96.7	3.3	0	0
AMK	100	0	0	0	98.6	1.4	0	0	90	10	0	0
CIP	100	0	0	0	98.6	1.4	0	0	96.7	3.3	0	0
Total	96.5	3.4	0.1	0	97.9	1.8	0.3	0	75.8	21.5	2.4	0.3

[a] AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin; PIP, piperacillin; TZP, piperacillin-tazobactam; CEF, cephalothin; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; FEP, cefepime; CAZ, ceftazidime; IPM, imipenem; ATM, aztreonam; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; CIP, ciprofloxacin.

misidentified by the Phoenix as *Streptococcus pneumoniae* (Table 2). The times required were 3 h 10 min, 3 h 30 min, 3 h, 2 h 30 min, and 3 h 30 min for a complete ID and 7 h 30 min, 16 h, 13 h, 10 h 30 min, and 11 h for ID plus AST for *E. coli*, all NFGNB, *P. aeruginosa* (exclusively), *S. aureus* and *E. faecalis*, respectively.

AST results. The AST results of all drug classes are shown in Tables 3 and 4. The overall agreement was 94.7%, the VME rate was 0.22%, the ME rate was 0.43%, and the mE rate was 4.65%. For gram-negative bacteria, the overall agreement rate was 91.4%, the VME rate was 0.08%, the ME rate was 0.80%, and the mE rate was 7.72%. For gram-positive bacteria, overall agreement rate was 98.66%, the VME rate was 0.39%, the ME rate was 0%, and the mE rate was 0.95%.

TABLE 4. Gram positive cocci: percentages of corresponding results between the Pheonix and agar diffusion methods

Antimicrobial agent	% Gram-positive											
	S. aureus (*n* = 42)				Other staphylococci (*n* = 31)				Enterococci (*n* = 22)			
	CA	mE	ME	VME	CA	mE	ME	VME	CA	mE	ME	VME
PEN[a]	97.7	2.3	0	0	93.6	3.2	0	3.2	100	0	0	0
OXA	97.7	0	0	2.3	96.8	0	0	3.2				
AMP									100	0	0	0
KAN	100	0	0	0	96.8	3.2	0	0				
TOB	100	0	0	0	100	0	0	0				
GEN	100	0	0	0	100	0	0	0	100	0	0	0
LIN	100	0	0	0	100	0	0	0	100	0	0	0
OFX	100	0	0	0	96.8	3.2	0	0				
VAN	100	0	0	0	100	0	0	0	95.5	4.5	0	0
TEC	100	0	0	0	90.4	9.6	0	0	100	0	0	0
RIF	100	0	0	0	100	0	0	0	100	0	0	0
COT	100	0	0	0	93.6	6.4	0	0	95.5	0	0	4.5
FSA	100	0	0	0	100	0	0	0				
TET	100	0	0	0	100	0	0	0				
Total	99.6	0.2	0	0.2	97.5	2.0	0	0.5	98.8	0.6	0	0.6

[a] PEN, penicillin; OXA, oxacillin; AMP, ampicillin; KAN, kanamycin; TOB, tobramycin; GEN, gentamicin; LIN, lincomycin; OFX, ofloxacin; VAN, vancomycin; TEC, teicoplanin; RIF, rifampin; COT, cotrimoxazole; FSA, fusidic acid; TET, tetracycline.

High mE rates were observed mainly for β-lactam antibiotics and gram-negative bacteria. For *E. coli* the mE rates for cephalothin and amoxicillin-clavulanic acid were 21 and 19%, respectively (Table 3). For *P. aeruginosa*, the VME and ME rates for ceftazidime were 3.4%, and the mE rates for ticarcillin, piperacillin, piperacillin-tazobactam, cefepime, ceftazidime, and aztreonam were 43.3, 23.3, 20, 30, 23.2, and 63.3%, respectively. For staphylococci, a VME rate of 0.33% was obtained due to a failure to detect the penicillin resistance in one strain and oxacillin resistance in two other strains, as confirmed by the detection of PBP2a. Staphylococci and enterococci produced mE rates for teicoplanin and vancomycin of 9.6 and 4.5%, respectively.

DISCUSSION

Current practice developed by technical staffs and microbiologists has led microbiology laboratories to rely on each individual's experience; these individuals use their own standards together with recommendations from scientific societies to interpret ID and AST results. The development of automated systems with in-built expert systems has allowed an increase both in the reproducibility and in the reliability of the results and consequently the quality of the results. These systems have also modified daily working practice, providing the ability to communicate early provisional results to the clinician. The workload demands in our microbiology laboratories have evolved dramatically, which, along with the fact that the measurement of zone sizes is tedious, time-consuming, and sensitive to transcription errors, has led us to promote the use of an automated system for ID and AST (the BD Phoenix system) with an associated software station (EpiCenter [BD]). ID and AST results obtained during the assessment study phase have confirmed robust performances of the Phoenix. Discrepant ID results by both methods were always confirmed by additional biochemical tests and 16S RNA sequencing (see Materials and Methods). The essential agreement rates for ID were nearly 92% for all isolates tested and 95.6, 100, and 100% for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. Mistakes or misidentifications observed with *E. coli* were essentially due to a software problem in the first version of the Phoenix system. This has forced us to use selective agar media to differentiate between lactose-positive and lactose-negative gram-negative bacilli for the control of the inoculum's purity. With the exception of the misidentifications observed with *E. coli* isolates and of a *C. brakii* identified instead of an *Enterobacter cloacae*, misidentifications were always at the species level only or in species for which too few representatives were tested for a rigorous assessment. Unambiguous separation of NFGNB, such as *Acinetobacter* spp. (2), requires a complex battery of phenotypic tests not often present in an ID panel. In addition, NFGNB represent several species for which a slow-growth or a mucous aspect is observed; this often represents a handicap for rapid ID methods (3, 9, 19).

Essential agreement rates for coagulase-negative staphylo-

cocci (CNS) and enterococci were 92 and 86%, respectively, findings comparable to previous results (10). Eight CNS and four enterococcus strains were misidentified by the Phoenix system. A first analysis showed that *E. faecalis* or *E. faecium* were frequently misidentified as *E. casseliflavus-gallinarum* when the inoculum was closer to 0.6 McFarland units compared to 0.5 McFarland units. Even if the phenotypic tests allowed the resolution of such a misidentification, the calibration of the inoculum at 0.5 McFarland units sharp is now recommended for gram-positive cocci. For CNS, similar levels of misidentification were observed, essentially due to the heterogeneity of the CNS population obtained from clinical specimens, i.e., similar colonies on a blood agar plate could correspond to different species for one clinical specimen (10). In addition, the fact that CNS were not identified as well as *S. aureus* might be explained by their slow metabolism, leading to ambiguous results in the reaction wells of the ID panel (32). Finally, the differentiation between *Streptococcus pneumoniae* and *Streptococcus mitis* by the Phoenix system is not optimal. One strain of *Streptococcus pneumoniae* was misidentified as *Streptococcus mitis* and vice versa for a second isolate. The number of tested strains was low, since the gram-positive Combo panel performed ID and AST only for enterococci. The development of a dedicated pneumococci Combo panel will perhaps resolve such discrepancies. Currently, it is essential in such conditions to check the inoculum on a blood agar plate with an Optochin disk and with the latex agglutination test for pneumococci.

Comparison of the AST results also demonstrated the performance of the Phoenix automatic system. Considering the total number of AST performed, an overall CA of nearly 95% was found, above the 90% minimal performance requirement (40). Similarly, low ME (0.43%) and VME (0.22%) rates, compared to the 3 and 1.5% established minimal performance requirements (40), were found. In addition, results were obtained in less time than by the disk diffusion method. No VME was observed, except for ceftazidime and *P. aeruginosa*, without any therapeutic consequences since, at the time of the study, only disk diffusion results were communicated to the clinician. The high rate of ME or mE for *P. aeruginosa* and β-lactam antibiotics such as ticarcillin, ticarcillin-clavulanic acid, and aztreonam was mainly due to improved detection of the efflux resistance phenotype (7, 19, 26) by the liquid medium. In comparison, MEs or mEs for piperacillin or piperacillin-tazobactam were due to a false interpretation of the presence of a penicillinase in such *P. aeruginosa* isolates with an efflux resistance phenotype not detected by the disk diffusion method. It is difficult to define reference phenotypes of resistance to beta-lactams in *P. aeruginosa* because the expression of the efflux pumps greatly influences the MICs of penicillins, cephalosporins, and carbapenems (30, 34). Moreover, the influence of other factors, such as altered PBPs, is largely unknown (5). Finally, a high ME rate for cefalothin and *E. coli* was observed, and similarly for amoxicillin-clavulanic acid. This was due to the detection of the low level of expression of the natural cephalosporinase (AmpC) from *E. coli* (20, 21, 28) in liquid medium.

The absence of VME for gram-positive cocci was observed, with the exception of staphylococci. These VMEs were observed mainly for oxacillin and in fact represent the limit of detection of the *mecA* resistance phenotype by the Phoenix automate and by other methods by using oxacillin as the key antibiotic for the detection of the methicillin resistance (10, 11, 44, 45).

In conclusion, the present study demonstrates the efficiency of the Phoenix system, which can be used for the majority of strains encountered in a university-based laboratory for ID and AST. Its association with an expert system and a database system has improved the workflow and the validation of AST results.

ACKNOWLEDGMENTS

We thank all of the members of the technical staff, without whom this study could not have been performed, and J. D. Cavallo for determining the MICs for the *P. aeruginosa* isolates. We are grateful to Ben Marshall (Southampton University Hospital, Southampton, United Kingdom) for carefully reading the manuscript and to Becton Dickinson for placing the Phoenix instrument at our disposal during the evaluation study and for providing the consumables.

REFERENCES

1. **Barry, J., A. Brown, V. Ensor, U. Lakhani, D. Petts, C. Warren, and T. Winstanley.** 2003. Comparative evaluation of the VITEK 2 Advanced expert system (AES) in five UK hospitals. J. Antimicrob. Chemother. **51:**1191–1202.
2. **Bouvet, P. J. M., and P. A. D. Grimont.** 1986. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov., and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffi.* Int. J. Syst. Bacteriol. **36:**228–240.
3. **Brisse, S., S. Stefani, J. Verhoef, A. Van Belkum, P. Vandamme, and W. Goessens.** 2002. Comparative evaluation of the BD Phoenix and VITEK 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. J. Clin. Microbiol. **40:**1743–1748.
4. **Carret, C., J. D. Cavallo, H. Chardon, C. Chidiac, P. Choutet, P. Courvalin, H. Dabernat, H. Drugeon, L. Dubreuil, F. Goldstein, V. Jarlier, R. Leclercq, M. H. Nicolas-Chanoine, A. Philippon, C. Quentin, B. Rouveix, J. Sirot, and J. C. Soussy.** 2003. Comite de l'Antibiogramme de la Societe Francaise de Microbiologie report 2003. Int. J. Antimicrob. Agents **21:**364–391.
5. **Cavallo, J. D., F. Leblanc, R. Fabre, A. Fourticq-Esqueöute, and the GERPB.** 2001. Survey of the antibiotic sensitivity of *Pseudomonas aeruginosa* in France and the distribution of beta-lactam resistance mechanisms: the GERPB 1999 study. Pathol. Biol. **49:**534–539.
6. **Chavez, M., J. L. Garcia-Lopez, J. Coronilla, A. Valverde, M. C. Serrano, R. Claro, and E. Martin-Mazuelos.** 2002. Evaluation of the new VITEK 2 system for determination of the susceptibility of clinical isolates of *Streptococcus pneumoniae*. Chemotherapy **48:**26–30.
7. **Dib, C., J. Trias, and V. Jarlier.** 1995. Lack of additive effect between mechanisms of resistance to carbapenems and other beta-lactams in *Pseudomonas aeruginosa.* Eur. J. Clin. Microbiol. Infect. **14:**979–986.
8. **Doern, G., R. Vautour, M. Gaudet, and B. Levy.** 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J. Clin. Microbiol. **32:**1757–1762.
9. **Endimiani, A., F. Luzzaro, A. Tamborini, G. Lombardi, V. Elia, R. Belloni, and A. Toniolo.** 2002. Identification and antimicrobial susceptibility testing of clinical isolates of nonfermenting gram-negative bacteria by the Phoenix automated microbiology system. New Microbiol. **25:**323–329.
10. **Fahr, A. M., U. Eigner, M. Armbrust, A. Caganic, G. Dettori, C. Chezzi, L. Bertoncini, M. Benecchi, and M. G. Menozzi.** 2003. Two-center collaborative evaluation of the performance of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of *Enterococcus* spp. and *Staphylococcus* spp. J. Clin. Microbiol. **41:**1135–1142.
11. **Felten, A., B. Grandry, P. H. Lagrange, and I. Casin.** 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J. Clin. Microbiol. **40:**2766–2771.
12. **Ferraro, M. J., and J. H. Jorgensen.** 1995. Instrument-based antibacterial susceptibility testing, p. 1375–1384. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
13. **Freeman, J. W., R. W. Rowland, S. B. Overman, and N. L. Goodman.** 1981. Laboratory evaluation of the AutoMicrobic system for identification of *Enterobacteriaceae*. J. Clin. Microbiol. **13:**895–898.
14. **Funke, G., C. Monnet, C. de Bernardis, A. von Graevenitz, and J. Freney.** 1998. Evaluation of the Vitek 2 system for rapid identification of medically relevant rods. J. Clin. Microbiol. **36:**1948–1952.

15. **Garcia-Garrote, F., E. Cercenado, and E. Bouza.** 2000. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. J. Clin. Microbiol. **38:**2108–2111.
16. **Hasyn, J. J., K. R. Cundy, C. C. Dietz, and W. Wong.** 1981. Clinical laboratory evaluation of the Auto-Microbic system for rapid identification of *Enterobacteriaceae*. J. Clin. Microbiol. **13:**491–497.
17. **Johnson, J. E., J. H. Jorgensen, S. A. Crawford, J. S. Redding, and R. C. Pruneda.** 1983. Comparison of two automated instrument systems for rapid susceptibility testing of gram-negative bacilli. J. Clin. Microbiol. **18:**1301–1309.
18. **Jossart, M. F., and R. J. Courcol.** 1999. Evaluation of an automated system for identification of *Enterobacteriaceae* and nonfermenting bacilli. Eur. J. Clin. Microbiol. Infect. Dis. **18:**902–907.
19. **Joyanes, P., M. del Carmen Conejo, L. Martínez-Martínez, and E. J. Perea.** 2001. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. J. Clin. Microbiol. **39:**3247–3253.
20. **Kazmierczak, A., X. Cordin, J. M. Duez, E. Siebor, A. Pechinot, and J. Sirot.** 1990. Differences between clavulanic acid and sulbactam in induction and inhibition of cephalosporinases in enterobacteria. J. Int. Med. Res. **18:**67–77.
21. **Kitzis, M. D., B. Ferre, A. Coutrot, J. F. Acar, and L. Gutman.** 1989. In vitro activity of combinations of beta-lactam antibiotics with beta-lactamase inhibitors against cephalosporinase-producing bacteria. Eur. J. Clin. Microbiol. Infect. Dis. **8:**783–788.
22. **Leclercq, R., M. H. Nicolas-Chanoine, P. Nordmann, A. Philippon, P. Marchais, A. Buu-Hoi, H. Chardon, H. Dabernat, F. Doucet-Populaire, C. Grasmick, P. Legrand, C. Muller-Serieys, J. Nguyen, M. C. Ploy, M. E. Reverdy, M. Weber, and R. J. Courcol.** 2001. Multicenter evaluation of an automated system using selected bacteria that harbor challenging and clinically relevant mechanisms of resistance to antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. **20:**626–635.
23. **Leverstein-van Hall, M. A., A. C. Fluit, A. Paauw, A. T. Box, S. Brisse, and J. Verhoef.** 2002. Evaluation of the Etest ESBL and the BD Phoenix, VITEK 1, and VITEK 2 automated instruments for detection of extended-spectrum beta-lactamases in multiresistant *Escherichia coli* and *Klebsiella* spp. J. Clin. Microbiol. **40:**3703–3711.
24. **Ligozzi, M., C. Bernini, M. Grazia Bonora, M. de Fatima, J. Zuliani, and R. Fontana.** 2002. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clin. Microbiol. **40:**1681–1686.
25. **Ling, T. K., P. C. Tam, Z. K. Liu, and A. F. Cheng.** 2001. Evaluation of VITEK 2 rapid identification and susceptibility testing system against gram-negative clinical isolates. J. Clin. Microbiol. **39:**2964–2966.
26. **Livermore, D. M.** 1992. Interplay of impermeability and chromosomal beta-lactamase in imipenem-resistant *Pseudomonas aeruginosa.* Antimicrob. Agents Chemother. **36:**2046–2048.
27. **McGregor, A., F. Schio, S. Beaton, V. Boulton, M. Perman, and G. Gilbert.** 1995. The MicroScan WalkAway diagnostic microbiology system: an evaluation. Pathology **27:**172–176.
28. **Moosdeen, F., J. Keeble, and J. D. Williams.** 1986. Induction / inhibition of chromosomal beta-lactamase inhibitors. Rev. Infect. Dis. **8:**S562–S568.
29. **Nadjar, D., M. Rouveau, C. Verdet, J. L. Donay, J. L. Herrmann, P. H. Lagrange, A. Philippon, and G. Arlet.** 2000. Outbreak of *Klebsiella pneumoniae* producing transferable AmpC-type β-lactamase (ACC-1) originating from *Hafnia alvei*. FEMS Microbiol. Lett. **187:**35–40.
30. **Nikaido, H.** 1998. Multiple antibiotic resistance and efflux. Curr. Opin. Microbiol. **1:**516–523.
31. **O'Hara, C. M., and J. M. Miller.** 2003. Evaluation of the Vitek 2 ID-GNB assay for identification of members of the family *Enterobacteriaceae* and other nonenteric gram-negative bacilli and comparison with the Vitek GNI+ card. J. Clin. Microbiol. **41:**2096–2101.
32. **Pfaller, M. A., and L. A. Herwaldt.** 1988. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. Clin. Microbiol. Rev. **1:**281–299.
33. **Pfaller, M. A., D. Sahm, C. O'Hara, C. Ciaglia, M. Yu, N. Yamane, G. Scharnweber, and D. Rhoden.** 1991. Comparison of the autoSCAN-W/A rapid bacterial identification system and the Vitek AutoMicrobic system for identification of gram-negative bacilli. J. Clin. Microbiol. **29:**1422–1428.
34. **Poole, K.** 2000. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob. Agents Chemother. **44:**2233–2241.
35. **Rhoads, S., L. Marinelli, C. A. Imperatrice, and I. Nachamkin.** 1995. Comparison of MicroScan WalkAway system and Vitek system for identification of gram-negative bacteria. J. Clin. Microbiol. **33:** 3044–3046.
36. **Sanders, C. C., M. Peyret, E. Smith Moland, S. J. Cavalieri, C. Shubert, K. S. Thomson, J. M. Boeufgras, and W. E. Sanders, Jr.** 2001. Potential impact of the VITEK 2 system and the Advanced Expert System on the clinical laboratory of a university-based hospital. J. Clin. Microbiol. **39:**2379–2385.
37. **Sanguinetti, M., B. Posteraro, T. Spanu, D. Ciccaglione, L. Romano, B. Fiori, G. Nicoletti, S. Zanetti, and G. Fadda.** 2003. Characterization of clinical isolates of *Enterobacteriaceae* from Italy by the BD Phoenix extended-spectrum β-lactamase detection method. J. Clin. Microbiol. **41:**1463–1468.
38. **Stager, C. E., and J. R. Davies.** 1992. Automated systems for identification of microorganisms. Clin. Microbiol. Rev. **5:**302–327.
39. **Tenover, F. C., T. S. Mizuki, and L. G. Carlson.** 1990. Evaluation of autoSCAN-W/A automated microbiology system for the identification of non-glucose-fermenting gram-negative bacilli. J. Clin. Microbiol. **28:**1628–1634.
40. **Thornsberry, C., J. P. Anhalt, J. A. Washington 2nd, L. R. McCarthy, F. D. Schoenknecht, J. C. Sherris, and H. J. Spencer.** 1980. Clinical laboratory evaluation of the Abbott MS-2 automated antimicrobial susceptibility testing system: report of a collaborative study. J. Clin. Microbiol. **12:**375–390.
41. **Thornsberry, C.** 1985. Automated procedures for antimicrobial susceptibility tests, p. 1015–1018. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Sadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
42. **van Den Braak, N., W. Goessens, A. van Belkum, H. A. Verbrugh, and H. P. Endtz.** 2001. Accuracy of the VITEK 2 system to detect glycopeptide resistance in enterococci. J. Clin. Microbiol. **39:**351–353.
43. **Woolfrey, B. F., R. T. Lally, and C. O. Quall.** 1983. Evaluation of the AutoSCAN-3 and Sceptor systems for *Enterobacteriaceae* identification. J. Clin. Microbiol. **17:**807–813.
44. **Yamazumi, T., S. A. Marshall, W. W. Wilke, D. J. Diekema, M. A. Pfaller, and R. N. Jones.** 2001. Comparison of the Vitek gram-positive susceptibility 106 card and the MRSA-screen latex agglutination test for determining oxacillin resistance in clinical bloodstream isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **39:**53–56.
45. **Yamazumi, T., I. Furuta, D. J. Diekema, M. A. Pfaller, and R. N. Jones.** 2001. Comparison of the Vitek gram-positive susceptibility 106 card, the MRSA-screen latex agglutination test, and *mecA* analysis for detecting oxacillin resistance in a geographically diverse collection of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol. **39:**3633–3636.