

## 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.

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The performance of the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, Md.) was assessed for identification (ID) and antimicrobial susceptibility testing (AST) for the majority of clinically encountered bacterial isolates in a European collaborative two-center trial. A total of 469 bacterial isolates of the genera *Staphylococcus* (275 isolates), *Enterococcus* (179 isolates), and *Streptococcus* (15 isolates, for ID only) were investigated; of these, 367 were single patient isolates, and 102 were challenge strains tested at one center. Sixty-four antimicrobial drugs were tested, including the following drug classes: aminoglycosides, beta-lactam antibiotics, beta-lactam-beta-lactamase inhibitors, carbapenems, cepheims, folate antagonists, quinolones, glycopeptides, macrolides-lincosamides-streptogramin B (MLS), and others. Phoenix ID results were compared to those of the laboratories' routine ID systems (API 32 Staph, API 32 Strep, and VITEK 2 [bioMérieux, Marcy l'Étoile, France]); Phoenix AST results were compared to those of frozen standard broth microdilution (SBM) panels according to NCCLS guidelines (NCCLS document M 100-S 9, approved standard M 7-A 4). Discrepant results were repeated in duplicate. Concordant IDs of 97.1, 98.9, and 100% were observed for staphylococci, enterococci, and streptococci, respectively. For AST results the overall essential agreement was 93.3%; the category agreement was 97.3%; and the very major error rate, major error rate, and minor error rate were 1.2, 1.9, and 1.3%, respectively. In conclusion, the Phoenix ID results showed high agreement with results of the systems to which they were being compared; the AST performance was highly equivalent to that of the SBM reference method.

The clinical microbiology laboratory is confronted with an alarming increase of antimicrobial resistance on a global scale (7, 8, 9, 13). Furthermore, the emergence of bacterial isolates with special resistance mechanisms such as oxacillin-resistant staphylococci or vancomycin-resistant enterococci constitutes a major problem, especially in intensive care units (1, 4). Both accurate and rapid diagnosis of oxacillin-resistant staphylococci and vancomycin-resistant enterococci has therefore become essential in the current health care environment.

The BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, Md. [BD]) is a newly developed instrument for the reliable and accurate identification and susceptibility testing for the majority of clinically encountered strains. The system is comprised of disposable panels, which combine both identification testing (ID) and antimicrobial susceptibility testing (AST), and an instrument which performs automatic reading at 20-min intervals during incubation. The system claims to provide accurate and rapid susceptibility results with easy workflow for the laboratory worker.

We report on the ability of the Phoenix system to accurately perform ID and AST of clinical and challenge isolates in a

large collaborative two-center trial involving the Section of Microbiology, University of Parma, Parma, Italy, and the Laboratory Group Heidelberg, Heidelberg, Germany. In this study, gram-positive bacteria were evaluated in a comparison of the system to routine laboratory methods for ID and to a standard broth microdilution (SBM) procedure for AST according to NCCLS guidelines (14).

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### MATERIALS AND METHODS

**Proficiency.** Each technician was required to simultaneously set up 20 strains (provided by the manufacturer) in both the Phoenix system and the reference AST system. Proficiency testing was successful if a correct result was obtained in 90% or more of single tests performed.

**Reproducibility.** This phase of the study was performed at one center (Parma). Fifteen strains (including the NCCLS-recommended quality control [QC] strains) provided by the manufacturer were set up on three different days in triplicate in the Phoenix system only. Results were evaluated to determine variability of repeat AST testing. The MIC results for each strain-antimicrobial agent combination were used to determine a modal MIC result, and the frequency of MICs within plus or minus one dilution of this mode was determined and used as an expression of reproducibility.

**Bacterial isolates.** A total of 469 bacterial isolates of the genera *Staphylococcus* (275 isolates), *Enterococcus* (179 isolates), and *Streptococcus* (15 isolates, evaluated for identification only) were investigated. The following species were included: *Staphylococcus aureus* (114 isolates), *Staphylococcus epidermidis* (90 iso-

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lates), *Staphylococcus haemolyticus* (23 isolates), *Staphylococcus capitis* (9 isolates), *Staphylococcus hominis* (8 isolates), *Staphylococcus saprophyticus* (6 isolates), *Staphylococcus warneri* (6 isolates), *Staphylococcus lugdunensis* (5 isolates), *Staphylococcus simulans* (4 isolates), *Staphylococcus cohnii* (4 isolates), other coagulase-negative staphylococci (CoNS) (6 isolates), *Enterococcus faecalis* (113 isolates), *Enterococcus faecium* (50 isolates), *Enterococcus gallinarum* (7 isolates), *Enterococcus casseliflavus* (5 isolates), other *Enterococcus* spp. (4 isolates), *Streptococcus agalactiae* (15 isolates). Of these strains, 367 were single patient isolates (200 from Heidelberg and 167 from Parma) and 102 were challenge strains supplied by the manufacturer to one of the sites (Heidelberg). The challenge set included strains from various sources, including the Centers for Disease Control and Prevention, French National Reference Center (Société Française de Microbiologie [SFM]), and BD internal collection with well-defined resistance mechanisms.

**Phoenix ID.** The Phoenix system used one ID and AST combination panel (CT04P), with the identification substrates on one side and antimicrobial drugs on the other side of the panel. The ID side of the panel for gram-positive bacteria contained a total of 45 dried substrates, including 20 fluorogenic substrates, 8 fermentation substrates, 8 carbon source substrates, 5 chromogenic substrates, esculin, urea, and two fluorescent controls. Isolates were subcultured twice onto Trypticase Soy Agar supplemented with 5% sheep blood (TSA II, BD Diagnostic Systems) to ensure viability and purity. The Phoenix ID broth was inoculated with bacterial colonies from a pure culture adjusted to a 0.5 to 0.6 McFarland standard using a CrystalSpec Nephelometer (BD Diagnostic Systems). After having transferred 25  $\mu$ l of the ID suspension to the Phoenix AST broth, the suspension was poured into the ID side of the Phoenix panel. Once inoculated the panel was logged and loaded into the instrument, where kinetic measurements of colorimetric and fluorescent signals were collected every 20 min.

**Reference ID.** The laboratory's routine ID system was set up from the same agar pure culture. In Heidelberg staphylococci were identified with the API 32 Staph system (bioMérieux, Marcy l'Etoile, France) and enterococci were identified with the API 32 Strep system (bioMérieux). At the University of Parma staphylococci and enterococci were investigated using the VITEK 2 system (bioMérieux).

Additionally, for staphylococci the clumping factor (Staphyslide; bioMérieux) and the coagulase test (rabbit plasma; bioMérieux) were used. For enterococci, the esculin reaction and, if necessary, the motility test were performed.

**Antimicrobials.** In total, 64 drugs were tested including the following drug classes (number of drugs): aminoglycosides (7), beta-lactam antibiotics (6), beta-lactam-beta-lactamase inhibitors (4), carbapenems (2), cepheems (17), folate antagonists (3), quinolones (10), glycopeptides (2), macrolides-lincosamides-streptogramin B (MLS) (7), and others (6). NCCLS breakpoints were utilized for most antimicrobial agents, but breakpoints from the Comité de l'Antibiogramme de la SFM (5) were used for four antibiotics (pristinamycin, pefloxacin, fusidic acid, and lincomycin) for which there are no NCCLS breakpoints. Additionally, breakpoints of the Deutsches Institut für Normung (DIN) (6) were used for trimethoprim-sulfamethoxazole utilizing the DIN recommended concentration range, and moxifloxacin was evaluated using breakpoints recommended by the pharmaceutical manufacturer (Bayer, Leverkusen, Germany, personal communication).

**Phoenix AST.** The Phoenix AST broth was supplemented by one drop of Phoenix AST indicator (oxidation-reduction indicator based on resazurin). From the standardized ID suspension 25  $\mu$ l was transferred to the AST broth, resulting in a final inoculum density of approximately  $5 \times 10^5$  CFU/ml. The broth was poured into the fill port on the AST side of the Phoenix panel. Following filling, the panels were sealed with a closure and together with three additional similarly inoculated AST-only panels (CT02P, CT03P, and CT11P) were logged and loaded into the Phoenix instrument. For each antibiotic a minimum of eight concentrations (doubling dilutions) were tested with the Phoenix system. The Phoenix panels contained a staphylococcal penicillinase test including a nitrocefin-based well on the ID side plus a growth based test for penicillinase production on the AST side. The two results were integrated into a single beta-lactamase test result. That is, if the beta-lactamase test was positive, the interpretation for penicillinase-susceptible penicillins (PNSP) was automatically set to resistant. The Phoenix penicillinase test was compared in this study to the cefinase test (BD Diagnostic Systems).

**Reference AST.** The reference method frozen SBM panels (five panels) contained the same antimicrobial agents in doubling dilutions as the Phoenix panels. The reference panels were prepared and tested according to NCCLS standards (14). The reference panel contained tests for high-level resistance to aminoglycosides (HLAR), including both gentamicin and streptomycin. Additionally, the following supplemental tests were performed: cefinase test without induction by oxacillin, catalase test, oxacillin screen agar (Oxascreeen; BD Diagnostic Sys-

tems), an *Enterococcus* QUAD plate containing 6  $\mu$ g of vancomycin, as well as gentamicin- and streptomycin-HLAR tests (BD Diagnostic Systems). The reference method for oxacillin was the SBM method for all staphylococci other than *S. aureus*, and SBM combined with the oxacillin screen agar was the reference method for *S. aureus*. When the oxacillin result was susceptible and the Oxascreeen result was positive, the reference oxacillin result was resistant.

**QC.** For QC, 16 ATCC strains were tested for each run, resulting in a total of 29 Phoenix and 41 reference panels: *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. faecalis* ATCC 14506, *E. faecalis* ATCC 49533, *E. faecalis* ATCC 10741, *E. faecium* SCPOS 4295, *E. faecalis* ATCC 51299, *S. aureus* ATCC 43300, *S. epidermidis* ATCC 35547, *S. saprophyticus* ATCC 35552, *Staphylococcus sciuri* ATCC 29062, *E. faecium* ATCC 49032, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 35218. For the reference system, results of QC strains had to be within the acceptable NCCLS limits, as defined by the U.S. Food and Drug Administration guidance document for AST devices (3, 14).

**Molecular tests.** For oxacillin-resistant staphylococci, a PCR-based *mecA* gene method was performed. The primers designed in our Laboratory Group were *Mec3s* (5'-ACA TCT ATT AGG TTA TGT TGG-3') and *Mec3as* (5'-TAT ATT CTT CGT TAC TCA TGC-3'), which produced a PCR product of 492 bp. For the PCR analysis of enterococcal *vanA*, *vanB*, and *vanC* gene clusters, primers described by Patel et al. were used; for detection, gel electrophoresis was performed without prior restriction enzyme digestion (15). Molecular methods were evaluated and discussed separately from the primary comparison of Phoenix to reference method.

**Data analysis and management.** The Phoenix and the reference data were entered into a Microsoft SQL Server (version 7.0) database (Microsoft Corporation, Redmond, Wash.). Applying NCCLS, SFM, DIN, or pharmaceutical company breakpoints and associated rule recommendations, sensitive, intermediate, and resistant (SIR) interpretations were determined electronically in the database for both the reference data and the Phoenix data, ensuring that the same rules were applied for each data set (9, 13). The Phoenix ID was used in the interpretation of all AST results obtained by both the Phoenix system and the SBM method for each respective isolate.

All AST accuracy reports were generated using SAS software (version 8.0; SAS Institute, Cary, N.C.). For each drug the following measures of accuracy were used: essential agreement (EA), or MICs between systems being within plus or minus one doubling dilution, and category agreement (CA), or SIR interpretative results matching between the two systems. Errors were classified as very major error (VME), or false susceptible Phoenix result; major error (ME), or false resistant Phoenix result; and minor error (mE), i.e., one system reporting an intermediate result and the other reporting a susceptible or resistant result. In calculating the error rates the following denominators have been used: the number of reference resistant isolates for VME rate, the number of reference susceptible isolates for ME rate, and the total number of tests for mE rate.

**Discrepancy resolution.** Isolates for which there were ID discrepancies, VME, and ME were subjected to repeat testing in duplicate in both the Phoenix system and the reference methods, giving three results in total. A majority rule determined the final resolved outcome. For remaining ID discrepancies both comparator methods (API 32 Staph, API 32 Strep, VITEK 2 [bioMérieux]) were set up.

## RESULTS

**Proficiency.** All laboratory personnel of both centers involved in this study passed the proficiency phase.

**Reproducibility.** On a total of 1,314 single tests, reproducibility testing within the expected modal MIC range showed correct results in 95.9% of cases. Reproducibility within the SIR categories showed correct results in 99.2% of cases.

**ID.** Out of 469 strains tested, a concordant ID to the species level was obtained in 97.9% of cases. Staphylococci, enterococci, and streptococci showed a concordant result in 97.1, 98.9, and 100% of cases, respectively; 10 strains, 6 clinical and 4 challenge isolates, showed an ID discordant with that of the respective comparator method (Table 1). For one challenge strain (*S. hominis*) the VITEK 2 comparator system also gave a discordant result, as did the Phoenix instrument (*S. haemolyticus*).

TABLE 1. Identification results for gram-positive cocci

Organism group	No. of isolates tested	No. (%) of IDs		Phoenix ID	n	Comparator system ID	
		Concordant	Discordant			API Staph or API Strep	VITEK 2
Staphylococcus	275	267 (97.1)	8 (2.9)	<i>Staphylococcus caprae</i> - <i>S. warneri</i> <sup>a</sup>	3	<i>S. cohnii</i>	<i>S. cohnii</i>
				<i>S. hominis</i> - <i>S. capitis</i>	2	<i>S. epidermidis</i>	<i>S. epidermidis</i>
				<i>S. haemolyticus</i>	1	<i>S. hominis</i>	<i>S. haemolyticus</i>
				<i>S. cohnii</i>	1	<i>S. saprophyticus</i>	<i>S. saprophyticus</i>
				<i>S. gallinarum</i>	1	<i>Staphylococcus xylosum</i>	<i>Staphylococcus xylosum</i>
Enterococcus	179	177 (98.9)	2 (1.1)	<i>E. casseliflavus</i> - <i>E. gallinarum</i>	2	<i>E. faecalis</i>	<i>E. faecalis</i>
Streptococcus	15	15 (100)					
Total	469	459 (97.9)	10 (2.1)				

<sup>a</sup> For *S. caprae*-*S. warneri*, n = 1; for *S. warneri*, n = 2.

**Overall AST results.** The total percentage of QC failures based on QC runs and single drug failures was 0.6%. A total of 22,300 single AST results were evaluated for staphylococci (17,481 results) and enterococci (4,819 results). The evaluation included 13,869 clinical and 3,612 challenge single test results for staphylococci and 3,851 clinical and 968 challenge single test results for enterococci. The AST results of all drug classes combined for Heidelberg and Parma are shown in Table 2. The overall EA was 93.3%, the CA was 97.3%, the VME rate was 1.2%, the ME rate was 1.7%, and finally, the mE rate was 1.3%. For staphylococci and enterococci the EA were 92.8 and 94.5%, the CA were 97.6 and 95.9%, the VME rates were 1.2 and 1.4%, the ME rates were 1.7 and 1.7%, and the mE rates were 0.9 and 2.7%, respectively.

**Staphylococcus AST results.** Table 3 shows the combined staphylococcus results of the individual drugs for both centers. Excluded from this table are the drugs for which there are no NCCLS, DIN, or SFM breakpoints available (5, 6, 14).

For penicillin and ampicillin the results for EA were below 90%, but CA results were greater than 95%. For penicillin the

ME rate was 24.1% and no VME was found. The high ME rate did not significantly affect the CA, because the frequency of susceptible strains was low compared to that of resistant strains. The absence of VME was also found for ampicillin while the ME rate was 7.1%.

The representative antibiotic for penicillinase-resistant penicillins (PNRP) in Phoenix is oxacillin. The EA for this drug was 94.2%, and the CA was 97.8%. Out of 116 *S. aureus* isolates tested, 54 were positive for the *mecA* gene. They were also oxacillin resistant according to the Phoenix system and the SBM method. Out of 161 CoNS tested, 155 strains gave the same results by all methods. For six CoNS isolates we found discrepant results; three CoNS were resistant according to the Phoenix system and the SBM method but did not express the *mecA* gene. For three strains VMEs were reported but the strains also lacked the *mecA* gene.

The glycopeptide antibiotics showed an EA of 96.4% and a CA of 99.6%; one intermediate and two resistant CoNS strains for teicoplanin were correctly determined by the Phoenix system. The MLS group showed an EA of 91.8% and a CA of

TABLE 2. Susceptibility test results for staphylococci and enterococci from Heidelberg and Parma

Organism group and center	No. of isolates tested	No. of single tests	No. of single tests in interpretive category <sup>a</sup>		Result (%)					
			I	R	EA	CA	mE	ME	VME	
Staphylococcus										
Parma	131	7,832	169	3,054	93.7	98.3	0.8	0.4	1.7	
Heidelberg	163	9,649	178	3,559	92.1	97.1	0.9	2.8	0.8	
Total	294	17,481	347	6,613	92.8	97.6	0.9	1.7	1.2	
Enterococcus										
Parma	58	1,694	203	553	94.8	96.3	2.6	1.3	1.1	
Heidelberg	106	3,125	327	1,198	94.4	95.7	2.7	1.9	1.5	
Total	164	4,819	530	1,751	94.5	95.9	2.7	1.7	1.4	
All										
Parma	189	9,526	372	3,607	94.0	98.0	1.1	0.5	1.6	
Heidelberg	269	12,774	505	4,757	92.8	96.8	1.3	2.6	1.0	
Total	458	22,300	877	8,364	93.3	97.3	1.3	1.7	1.2	

<sup>a</sup> Abbreviations: I, intermediate; R, resistant.

TABLE 3. Susceptibility test results for staphylococci

Class and drug	No. of isolates	Breakpoint standard	No. in interpretive category <sup>a</sup>			Result (%)				
			S	I	R	EA	CA	mE	ME	VME
Glycopeptides										
Vancomycin	286	NCCLS	286			97.6				
Teicoplanin	275	NCCLS	272	1	2	95.3	100.0	0.0	0.0	0.0
MLS										
Azithromycin	272	NCCLS	128	9	135	87.9	93.0	2.2	10.2	0.0
Clindamycin	288	NCCLS	207	5	76	96.5	97.2	1.4	1.9	0.0
Clarithromycin	285	NCCLS	143	1	141	91.9	95.8	0.4	7.7	0.0
Lincomycin	281	SFM	192	6	83	93.6	97.2	0.7	3.1	0.0
Pristinamycin	287	SFM	274	11	2	92.0	95.5	4.2	0.4	0.0
Quinupristin-dalfopristin	288	NCCLS	287		1	86.8	98.3	0.7	1.0	0.0
Erythromycin	265	NCCLS	126	12	127	93.6	93.6	6.4	0.0	0.0
Penicillins										
Ampicillin	274	NCCLS	56		218	67.7	98.5	0.0	7.1	0.0
Penicillin	273	NCCLS	54		219	74.2	95.2	0.0	24.1	0.0
PNRP (oxacillin)	277	NCCLS	128		149	94.2	97.8	0.0	2.3	2.0
$\beta$ -Lactam- $\beta$ -lactamase inhibitor										
Amoxicillin-clavulanate	535	NCCLS	249		286	95.7	97.8	0.0	2.4	2.1
Ampicillin-sulbactam	274	NCCLS	126		148	94.7	97.8	0.0	2.4	2.0
Piperacillin-tazobactam	270	NCCLS	124		146	94.6	97.8	0.0	2.4	2.1
Ticarcillin-clavulanate	264	NCCLS	55		209	88.8	98.9	0.0	5.5	0.0
Carbapenems										
Imipenem	273	NCCLS	127		146	98.4	97.8	0.0	2.4	2.1
Meropenem	272	NCCLS	127		145	94.6	97.8	0.0	2.4	2.1
Cephems										
Cefazolin	271	NCCLS	124		147	93.0	97.4	0.4	2.4	2.0
Cefdinir	267	NCCLS	124		143	90.5	96.3	0.7	4.0	2.1
Cefepime	272	NCCLS	125		147	95.2	97.8	0.0	2.4	2.0
Cefmetazole	272	NCCLS	125		147	93.0	97.8	0.0	2.4	2.0
Cefoperazone	271	NCCLS	126		145	93.0	97.8	0.0	2.4	2.1
Cefotaxime	274	NCCLS	128		146	90.6	97.8	0.0	2.3	2.1
Cefotetan	264	NCCLS	121	4	139	86.3	97.7	0.4	2.5	1.4
Cefoxitin	269	NCCLS	126	1	142	93.7	97.0	1.1	2.4	1.4
Cefpodoxime	273	NCCLS	86	40	147	93.8	96.7	1.5	3.5	1.4
Ceftazidime	272	NCCLS	106	20	146	91.3	96.7	1.8	1.9	1.4
Ceftizoxime	268	NCCLS	125	2	141	91.0	97.4	0.4	2.4	2.1
Ceftriaxone	273	NCCLS	127		146	90.2	97.1	0.4	3.9	1.4
Cefuroxime	268	NCCLS	128		140	97.7	97.8	0.0	2.3	2.1
Cephalothin	276	NCCLS	128		148	95.3	97.1	0.4	2.3	2.7
Cefaclor	269	NCCLS	125		144	92.1	97.4	0.0	4.0	1.4
Cephalexin	271		127		144	80.3	96.7	0.0	4.7	2.1
Aminoglycosides										
Amikacin	290	NCCLS	268	8	14	82.4	95.2	3.8	1.1	0.0
Gentamicin	288	NCCLS	199	5	84	96.2	98.6	0.3	1.0	1.2
Kanamycin	285	NCCLS	169	1	115	95.4	98.2	1.1	1.2	0.0
Netilmicin	289	NCCLS	279	6	4	93.1	97.9	1.0	1.1	0.0
Tobramycin	288	NCCLS	176	10	102	83.3	97.9	1.7	0.6	0.0
5-Fluoroquinolones										
Ciprofloxacin	289	NCCLS	179	4	106	97.6	99.0	0.0	0.0	2.8
Gatifloxacin	285	NCCLS	219	55	11	97.9	100.0	0.0	0.0	0.0
Grepafloxacin	285	NCCLS	184	1	100	96.1	96.8	2.5	0.5	1.0
Levofloxacin	285	NCCLS	185	27	73	98.2	98.2	1.4	0.0	1.4
Lomefloxacin	290	NCCLS	173	13	104	97.6	99.7	0.3	0.0	0.0
Moxifloxacin	286	PHARM <sup>b</sup>	262	17	7	97.9	98.6	1.0	0.4	0.0
Norfloxacin	287	NCCLS	175	7	105	93.7	99.3	0.7	0.0	0.0
Oloxacin	290	NCCLS	187		103	99.0	99.3	0.3	0.0	1.0
Trovafloxacin	289	NCCLS	258	19	12	96.5	98.3	1.7	0.0	0.0
Pefloxacin	287	SFM	165	14	108	98.6	99.0	0.7	0.0	0.9
Folate antagonists										
Trimethoprim	279	NCCLS	214		65	91.0	97.8	0.0	2.8	0.0
Trimethoprim-sulfamethoxazole (SXT)	279	NCCLS	235		44	90.7	96.8	0.0	2.6	6.8
Trimethoprim-sulfamethoxazole (STG)	263	DIN	221	11	31	92.4	94.7	3.4	1.8	3.2
Others										
Tetracycline	287	NCCLS	237	10	40	91.6	95.5	2.8	2.1	0.0
Chloramphenicol	288	NCCLS	251	8	29	89.9	95.5	3.8	0.8	0.0
Nitrofurantoin	287	NCCLS	287			100.0	100.0	0.0	0.0	0.0
Fusidic acid	287	SFM	263	16	8	95.1	97.2	2.8	0.0	0.0
Linezolid	265	NCCLS	265			96.6	100.0	0.0	0.0	0.0
Rifampin	288	NCCLS	268	3	17	97.6	99.3	0.7	0.0	0.0

<sup>a</sup> Abbreviations: S, susceptible; I, intermediate; R, resistant.<sup>b</sup> Pharmaceutical company.

TABLE 4. Susceptibility test results for enterococci

Class and drug	No. of isolates	Breakpoint standard	No. in interpretive category <sup>a</sup>			Result (%)				
			S	I	R	EA	CA	mE	ME	VME
Glycopeptides										
Vancomycin	163	NCCLS	131	4	28	98.2	98.8	0.6	0.8	0.0
Teicoplanin	163	NCCLS	145		17	98.8	99.4	0.0	0.7	0.0
MLS										
Pristinamycin	163	SFM	68	61	34	90.2	93.9	5.5	1.5	0.0
Quinupristin-dalfopristin	164	NCCLS	50	20	94	93.3	94.5	1.8	12.0	0.0
Erythromycin	146	NCCLS	16	60	70	88.4	89.7	10.3	0.0	0.0
$\beta$ -Lactam penicillins										
Ampicillin	163	NCCLS	129		34	98.2	99.4	0.0	0.8	0.0
Penicillin	163	NCCLS	120		43	96.9	100.0	0.0	0.0	0.0
Aminoglycosides										
Gentamicin (synergy)	163	NCCLS	115		48	100.0	98.2	0.0	0.9	4.2
Streptomycin (synergy)	163	NCCLS	105		58	100.0	98.2	0.0	0.0	5.2
5-Fluoroquinolones										
Ciprofloxacin	162	NCCLS	67	30	65	92.6	92.6	3.7	0.0	9.2
Levofloxacin	161	NCCLS	105	10	46	96.3	98.1	1.9	0.0	0.0
Trovafloxacin	160	NCCLS	112	14	34	88.8	92.5	6.3	0.0	5.9
Pefloxacin	162	SFM	1	85	76	92.6	92.6	6.8	0.0	1.3
Norfloxacin	161	NCCLS	89	22	50	94.4	96.3	3.7	0.0	0.0
Others										
Tetracycline	163	NCCLS	60	5	98	96.3	96.3	1.8	3.3	0.0
Chloramphenicol	162	NCCLS	129	9	24	93.2	96.9	1.2	2.3	0.0
Nitrofurantoin	163	NCCLS	120	39	14	98.7	98.8	1.2	0.0	0.0
Linezolid	146	NCCLS	146			98.0	100.0	0.0	0.0	0.0
Rifampin	163	NCCLS	56	40	67	68.1	76.1	13.5	30.4	0.0

<sup>a</sup> Abbreviations: S, susceptible; I, intermediate; R, resistant.

95.8%, and no VMEs were detected. The EA for linezolid was 96.6%, and all strains were susceptible by both methods.

For the beta-lactam-beta-lactamase inhibitor combinations only ticarcillin-clavulanate had an EA of 88.8%; the other four formulations showed values of 94.6 to 95.7%. For imipenem and meropenem the EAs were 98.4 and 94.6%, respectively. For the 17 cepheims tested in this trial, the EAs ranged from 90.2 to 97.7%, except for cephalixin (80.3%) and cefotetan (86.3%). The three VMEs observed with each of these drugs resulted from the resistance mechanism to oxacillin.

Among the aminoglycosides only amikacin and tobramycin had an EA of 82.4 and of 83.3%, whereas all other drugs had values above 90%; all drugs of this class showed a CA of 95.2 to 98.6%, and only one VME was seen, with gentamicin. The results of the EA of the 5-fluoroquinolones showed a distribution from 93.7 to 99.0%; the CA ranged from 96.8 to 100%, and the overall VME rate was 1.0%.

**Enterococcus AST results.** The results for enterococci are shown in Table 4. Excluded from this table are the drugs for which there are no NCCLS, DIN, or SFM breakpoints available.

For the glycopeptides the EA was 98.5% and the CA was 99.1%, with a VME rate of 0% and an ME rate of 0.7%. All of the 32 vancomycin-intermediate or -resistant *E. faecalis* and *E. faecium* isolates were detected by the Phoenix system, while one *E. faecium* isolate gave a false resistant result. Ampicillin and penicillin showed an EA of 98.2 and 96.9%, a CA of 99.4

and 100%, and a VME rate of 0 and 0%, respectively. The CA of the high-level resistance for gentamicin and streptomycin was 98.2%, with a VME rate of 4.7%. The five 5-fluoroquinolones showed an EA of 93.0%, a CA of 94.4%, and a VME rate of 3.3%. The EA for linezolid was 98.0%; all strains were susceptible by both methods.

## DISCUSSION

This two-center trial is focused on the performance of the Phoenix system with gram-positive strains. Prior to this report, only one such study had been published, by Brisse et al. (2), but numerous posters have been presented on the comparison between the Phoenix system and other reference and commercial ID and AST systems. We have compared the ID performance of the Phoenix to commercially available ID methods routinely used in our laboratories. In this evaluation only 10 out of 469 gram-positive strains tested showed an incorrect ID result; eight were CoNS strains, and no *S. aureus* isolate was misidentified. The discrepancies concerning two enterococcal isolates were resolved with the motility test. This finding was also reported with the VITEK 2 system by Garcia-Garrote et al., where 10 out of 55 *E. faecium* isolates with low-level resistance to vancomycin were identified as *E. gallinarum*-*E. casseliflavus* and where the motility test also resolved these discrepancies (10). When testing more than 1,000 gram-positive isolates, Salomon et al. could demonstrate the discriminatory

power of the different substrate classes used in the Phoenix system (J. E. Salomon, T. Wiles, C. Yu, and T. Dunk, Abstr. 99th Gen. Meet. Am. Soc. Microbiol. 1999, abstr. C-448, 1999). This resulted in a list of approximately 100 gram-positive species (taxon list) which can be identified by the Phoenix system. Our testing included only a small proportion of this taxon list; however, this challenge represented the most frequently encountered species in a routine clinical laboratory. Marco et al. investigated 136 gram-positive cocci with the Phoenix instrument and the MicroScan Walk-Away-40 (Dade-MicroScan, W. Sacramento, Calif.) and reported a concordance with this MicroScan system of 98.5% including arbitration (F. Marco, A. Jurado, and M. T. Jimenez de Anta, Abstr. 12th Eur. Cong. Clin. Microbiol. Infect. Dis. 2002, abstr. P 711, 2002). When comparing the performance of the Phoenix instrument with the VITEK 2 system Gross et al. investigated 400 staphylococcal strains and 121 *Enterococcus* spp. (R. Gross, U. Hörling, and G. Peters, Abstr. 12th Eur. Cong. Clin. Microbiol. Infect. Dis. 2002, abstr. P 703, 2002). Out of 520 gram-positive strains tested, 498 gave similar ID results in both systems. Most of the discrepant results occurred with CoNS.

We have compared the AST performance of the Phoenix system to the broth microdilution reference method with a broad range of antimicrobial agents. The PNSP class of antibiotics is one of the clinically important drug classes for enterococci which to a lesser extent is also true for staphylococci. For enterococci the results of EA, CA, VME, ME, and mE for all PNSP were very good. For ampicillin, which is generally considered to be an indicator antibiotic for enterococci, there were zero VME out of 32 resistant strains. This has also been confirmed by other groups (T. Wiles, W. Brasso, D. Turner, D. Holliday, and K. Fischbein, Abstr. 9th Eur. Cong. Clin. Microbiol. Infect. Dis. 1999, abstr. P 1156, 1999; F. Marco, A. Jurado, and M. T. Jimenez de Anta, Abstr. 12th Eur. Cong. Clin. Microbiol. Infect. Dis. 2002, abstr. P 712, 2002; R. Gross, U. Hoerling, and G. Peters, Abstr. 12th Eur. Cong. Clin. Microbiol. Infect. Dis. 2002, abstr. P 704, 2002).

For staphylococci we observed a low EA for penicillin (74.2%) and ampicillin (67.7%), while the CA was 95.2 and 98.5%, respectively. The integration of the penicillinase test into the interpretation of the penicillin MIC resulted in the high CA by virtually eliminating false susceptible results. This was also found by Wiles et al., who found the EAs to be 85% (penicillin) and 86% (ampicillin) (Wiles et al., 9th ECCMID) but found high CAs of 99 and 97%, respectively. Marco et al. also reported a Phoenix CA of 93.7% in comparison to the MicroScan system result for penicillin, and Gross et al. found a 99.1% Phoenix CA in comparison to the VITEK 2 system (Marco et al., 12th ECCMID, abstr. P 712; Gross et al., 12th ECCMID, abstr. P 704). In this study we observed no VME for any PNSP but an ME rate with penicillin of 24.1%. Yu et al. investigated 95 staphylococcal isolates for beta-lactamase activity, comparing the Phoenix system with the Cefinase Plus disk (BD Diagnostic Systems), which is reported to be more sensitive than the cefinase test which we used in our protocol (C. Yu, D. Turner, G. Karr, J. Sinha, and S. Wulff, Abstr. 9th Eur. Cong. Clin. Microbiol. Infect. Dis. 1999, abstr. P 38, 1999). This study did not use the above product because it had been discontinued by BD prior to our study. They also tested for beta-lactamase production following induction with oxacil-

lin and reported that 13 of these strains were determined to be sensitive to penicillin using the SBM, where these were positive by the Cefinase Plus disk procedure and thus should be resistant to all PNSP. It is possible that some of the Phoenix MEs which we observed are truly cases of PNSP resistance in which the reference cefinase did not detect beta-lactamase due to the fact that this study used the less-sensitive indicator and did not involve testing with induction.

Arguably the most significant AST test for staphylococcus is with the PNRP, which in the case of Phoenix is represented by oxacillin. This test is crucial because it identifies the critical resistance mechanism of methicillin-resistant staphylococcus (MRS), which is related to the acquisition of a modified PBP 2a encoded by the *mecA* gene. The presence of this resistance mechanism renders or implies resistance to all current beta-lactam antibiotics (according to NCCLS, SFM, and DIN breakpoints). However, many of these other beta-lactam antibiotics test in vitro as susceptible. Thus, the test of one PNRP influences the results for many antibiotics. The performance of the Phoenix system with oxacillin was an area of great interest in our study. We observed very good performance with only three VMEs out of 149 MRS strains tested. These VMEs occurred with CoNS only in cases where definition of MIC breakpoints is currently controversial and changing. The overall EA and CA were excellent, at 94.2 and 97.8%, respectively. Similar performance was observed by other groups (C. Yu, W. Brasso, D. Holliday, B. Turng, and J. Sinha, Abstr. 9th Eur. Cong. Clin. Microbiol. Infect. Dis. 1999, abstr. P 37; Gross et al., 12th ECCMID, abstr. P 704; J. A. Johnson, P. Murray, G. A. Denys, K. C. Hazen, and M. Saubolle, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-118, 2002; Marco et al., 12th ECCMID, abstr. P 712; D. M. Silver, L. Louie, and A. E. Simor, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-132, 2002). Silver et al. observed for staphylococci a slightly higher VME rate of 9.8% for the Phoenix system and of 8.1% for the VITEK 2 system, mostly seen with isolates that had low-level oxacillin resistance (MICs between 4 and 8 µg/ml) (Silver et al., 102nd Gen. Meet. Am. Soc. Microbiol.). Conversely, Johnson et al. detected 98.1% out of 312 methicillin-resistant *S. aureus* isolates tested (VME, 1.9%) with the Phoenix system when compared to SBM (Johnson et al., 102nd Gen. Meet. Am. Soc. Microbiol.). Likewise this performance is equivalent to those of the VITEK 2 and MicroScan systems overnight (12, 16, 17).

Given the current controversy regarding oxacillin breakpoints with CoNS, we tested all oxacillin-resistant strains for the *mecA* gene and found 3 out of 95 isolates to be *mecA* gene negative. Three additional *mecA*-negative strains tested oxacillin sensitive by the Phoenix but resistant by the SBM method. This finding was confirmed by Horstkotte et al., who tested 124 CoNS strains by the Phoenix system and compared the results to *mecA* gene results (M. A. Horstkotte, J. Knobloch, H. Rohde, and D. Mack, Abstr. Dtsch. Gesellsch. Hyg. Mikrobiol. 2000, abstr. P 020, 2002). Using the new NCCLS breakpoints they found only one VME but 26 MEs; 15 of these 26 were non-*S. epidermidis* strains. However, when applying the DIN breakpoint of 2.0 µg/ml, only four MEs would have been reported. Marco et al. and Gross et al. also questioned the appropriateness of the new NCCLS breakpoint for CoNS other than *S. epidermidis*. (Marco et al., 12th ECCMID, abstr.

P 712; Gross et al., 12th ECCMID, abstr. P 704). Similar conclusions have been stated by Hussain et al. (11). Taking into account both the issues with the breakpoints and the reported results, we conclude that the Phoenix system gives very satisfying oxacillin test results.

The most commonly used antibiotic for MRS is vancomycin. With this antimicrobial agent, we observed a very good EA of 97.6%. The CA was also very good. Outright resistance to vancomycin has only been reported with staphylococci very recently, in two patients from geographically widely separated areas in the United States (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5126a1.htm> and <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5140a3.htm>). In both cases an enterococcus *vanA* gene was detected. An intermediate level of resistance has previously been reported rarely worldwide for *S. aureus*, *S. haemolyticus*, and *S. epidermidis*. Such strains were not available in this study.

Vancomycin is the antibiotic of choice for serious infections with ampicillin-resistant enterococci. Enterococcal resistance to vancomycin is commonly observed in the United States, and its frequency is increasing throughout the world (1, 4). All of the 32 vancomycin-resistant enterococci were correctly detected by the Phoenix system, and there was only one false resistant result. Gross et al. and Marco et al. found a CA of 100% for enterococci and vancomycin (Gross et al., 12th ECCMID, abstr. P 704; Marco et al., 12th ECCMID, abstr. P 712). Butterworth et al. investigated 86 isolates of *E. faecium* (46 with the *vanA* gene and 22 with the *vanB* gene) (A. M. Butterworth, B. Turng, M. Votta, T. Wiles, J. Salomon, and J. Reuben, Abstr. 12th Eur. Cong. Clin. Microbiol. Infect. Dis. 2002, abstr. P 706). Compared to molecular methods the Phoenix system and the SBM method gave equal results, detecting 95% (44 of 46) of the *vanA* strains and 77% (17 of 22) of the *vanB* strains; for the latter the Bauer-Kirby method gave a performance of 100%, and for the *vanA* strains it gave a performance of 87%. With 732 clinical enterococcal strains tested by the Phoenix system and SBM method for vancomycin resistance, Hamel et al. found a sensitivity and specificity of 100 and 98.7%, respectively, with no VME (K. M. Hamel, G. A. Denys, K. C. Hazen, P. Murray, J. Johnson, and M. Saubolle, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-118, 2002).

The MLS class is of some therapeutic importance for staphylococci but limited importance for enterococci, except for quinupristin-dalfopristin with *E. faecium*. For staphylococci the CA ranged from 93.0% (azithromycin) to 98.3% (quinupristin-dalfopristin); a CA of 94.5% was observed for enterococci with quinupristin-dalfopristin. Reuben et al. described for both bacterial groups a CA of 95, 99, and 98% for azithromycin, clarithromycin, and quinupristin-dalfopristin, respectively, with one VME and three MEs (J. Reuben, D. Turner, C. Yu, and T. Wiles, Abstr. 40th Intersci. Conf. Antimicrobial Agents and Chemother., abstr. 1621, 2000).

Among the aminoglycosides only gentamicin results for staphylococci are available from other studies. Wiles et al. found an EA of 95% and a CA of 96% with no VME (Wiles et al., 9th ECCMID). These results are confirmed by Gross et al. (CA, 98.2%), Marco et al. (CA, 100%), and in the present study with 98.6% (Gross et al., 12th ECCMID, abstr. P 704; Marco et al., 12th ECCMID, abstr. P 712).

For HLAR of enterococci we detected two VMEs for gentamicin synergy and three VMEs for streptomycin synergy out of 163 isolates tested (combined VME, 4.72%), with a CA of 98.2%. Of these total five VMEs the SBM resistance result was verified by agar screen for three strains. Compared to the SBM results, Hong et al. detected no VME for both drugs out of 45 enterococci tested (J. Hong, J. Hejna, B. Turng, and V. Kennedy, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. C-308, 2000). Gross et al. found three major discrepancies (90 strains tested), and Marco et al. detected no major differences for HLAR (40 strains tested) (Gross et al., 12th ECCMID, abstr. P 704; Marco et al., 12th ECCMID, abstr. P 712).

For the 5-fluoroquinolones there were a high EA and a high CA for staphylococci in our study, which was confirmed by the other working groups for ciprofloxacin (Wiles et al., 9th ECCMID; Gross et al., 12th ECCMID, abstr. P 704; Marco et al., 12th ECCMID, abstr. P 712).

In conclusion, the ID results of the Phoenix system were in very high agreement with those of the commercially available comparator systems used in this study. The AST performance with *Staphylococcus* spp. and *Enterococcus* spp. was generally highly equivalent to that of the SBM method. The specificity of the system, i.e., detection of susceptible strains, was, with a few exceptions, also very good. The Phoenix system was very accurate in detecting the most important resistance mechanisms encountered by this group of microorganisms. This included oxacillin resistance for staphylococci and vancomycin resistance for enterococci, compared to molecular methods.

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