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Evaluation of the Merlin MICRONAUT System for Rapid Direct Susceptibility Testing of Gram-Positive Cocci and Gram-Negative Bacilli from Positive Blood Cultures[▽]

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Bloodstream infections are life-threatening conditions which require the timely initiation of appropriate antimicrobial therapy. We evaluated the automated Merlin MICRONAUT system for rapid direct microtiter broth antimicrobial susceptibility testing (AST) of gram-positive cocci and gram-negative bacilli from BACTEC 9240 bottles with positive blood cultures in comparison to the standard method for the Merlin MICRONAUT system. This prospective study was conducted under routine working conditions during a 9-month period. Altogether, 504 isolates from 409 patients and 11,819 organism-antibiotic combinations were evaluated for comparison of direct and standard AST methods. For gram-negative bacilli, direct and standard AST of 110 isolates was evaluated and MIC agreement was found for 98.1% of 2,637 organism-antibiotic combinations. Category (susceptible, intermediate susceptible, resistant [SIR]) agreement was found for 99.0%, with results for 0.04% of combinations showing very major errors, those for 0.2% showing major errors, and those for 0.8% showing minor errors. For gram-positive cocci, 373 isolates were evaluated and MIC agreement was found for 95.6% of 8,951 organism-antibiotic combinations. SIR agreement was found for 98.8%, with results for 0.3% of combinations showing very major errors, those for 0.4% showing major errors, and those for 0.5% showing minor errors. Although the number of tested isolates was limited ($n = 33$), direct AST of streptococci was performed for the first time, yielding promising results with SIR agreement for 98.6% of 363 organism-antibiotic combinations. In conclusion, direct AST of gram-negative bacilli and gram-positive cocci from positive blood cultures with the MICRONAUT system is a reliable technique that allows for the omission of repeat testing of subcultured isolates. Thereby, it reduces the time to results of blood culture testing and may have a positive impact on patient care.

Bloodstream infections are life-threatening conditions which require the timely initiation of antimicrobial therapy. Inappropriate initial antimicrobial therapy of septic patients is associated with adverse outcomes (13, 15, 20). Automated blood culture systems that monitor blood culture bottles continuously for bacterial growth minimize the time necessary to detect positive blood cultures. Once bacterial growth is detected in blood cultures, rapid identification and susceptibility testing of the isolate are important tasks for the clinical microbiology laboratory. Reducing the turnaround time of microbiological analysis by using automated systems can lead to significant reductions in patient morbidity, mortality, and costs (3, 9, 27).

While standard antimicrobial susceptibility testing (AST) of bacteria commonly involves pure overnight subcultures, preparation of the inoculum for automated susceptibility testing directly from the positive blood culture appears extremely attractive with respect to the time to results. Thus, direct antimicrobial susceptibility testing of isolates from positive blood cultures with many automated testing systems, like the Phoenix (BD, Heidelberg, Germany), the VITEK and VITEK 2 (BioMérieux, Nürtingen, Germany), the Sensititre (Trek Diagnostics, West Lake, OH), and the MicroScan (Dade Behring, Eschborn, Germany) systems, has been evaluated previously (4–6, 12, 14, 18, 21, 24, 26, 28). In general, good agreement between direct and standard susceptibility testing results was observed when gram-negative bacilli were tested, including both members of the *Enterobacteriaceae* and *Pseudomonas* species (4–6, 12, 14, 18, 21, 24, 26, 28). For direct testing of gram-positive cocci from blood cultures, only limited data from small studies are available for the VITEK, the VITEK 2, the Sensititre, and the MicroScan systems (5, 6, 8, 18, 26, 29). A significantly higher rate of disagreement between direct and standard testing results for gram-positive cocci than for gram-negative bacilli was found. Reporting of false susceptibility of staphylococci to oxacillin and of enterococci to various antibiotics (18, 26) is a major problem with enormous clinical relevance. Since gram-positive cocci cause the majority of bloodstream infections (23, 29), rapid and reliable automated susceptibility testing of gram-positive bacteria is highly desirable.

We evaluated the automated MICRONAUT system (Merlin, Bornheim-Hesel, Germany) for rapid direct microtiter broth susceptibility testing of gram-positive cocci and gram-negative bacilli from BACTEC bottles with positive blood cultures. The study was conducted under routine working conditions in the clinical microbiological laboratory of the University Hospital of Ulm, Ulm, Germany, during a 9-month period and included 850 positive blood cultures.

MATERIALS AND METHODS

Samples. The study was conducted from July 2005 to March 2006 at the University Hospital of Ulm, a 1,100-bed tertiary-care hospital which provides a

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full range of medical and surgical services. The automated blood culture system BACTEC 9240 (BD) with the culture bottles PLUS Aerobic/F, PLUS Anaerobic/F, and PLUS Pediatric is used in the hospital. One blood culture consists of an aerobic and an anaerobic bottle or, in the case of children, only a pediatric bottle. All blood cultures that were detected as positive by the BACTEC system and that showed gram-positive cocci or gram-negative bacilli in at least one bottle in the initial Gram staining were included in the study. If samples in both the aerobic and anaerobic bottles for one blood culture were detected as positive and the organisms showed identical Gram staining morphologies, only the aerobic bottle was used for the study. Blood cultures showing mixed growth in the initial Gram staining, i.e., more than one morphology of bacteria in a single bottle, were excluded from the study. The study was conducted on both weekdays and weekends. If isolates of the same species with identical antimicrobial susceptibility testing profiles were detected in more than one blood culture within 14 days, the direct susceptibility testing of the first isolate only was repeated by the standard method and results for the subsequent isolates were not included in the final data analyses (see below).

Standard susceptibility testing. Standard testing of all isolates was performed with a pure overnight subculture with the MICRONAUT system as recommended by the manufacturer (Merlin). The MICRONAUT system is an automated microtiter broth dilution susceptibility testing system that is distributed throughout Germany and Europe in private and hospital-based laboratories. The testing is performed with 384-well microtiter plates. This system allows the determination of real MICs of up to 25 substances and the testing of two bacterial isolates on one plate. Bacterial growth in the wells is monitored photometrically at a wavelength of 620 nm, and a density above the cutoff value for the respective medium is interpreted to indicate bacterial growth. Several colonies were used to prepare a 0.5-McFarland-standard suspension in 0.9% saline. For the testing of staphylococci, enterococci, and micrococci, 100 μl of the suspension was diluted with 15 ml of Mueller-Hinton II broth (containing 0.25 g/liter phytagel, an agar substitute produced from bacterial fermentation [Oxoid, Wesel, Germany]), while for the testing of gram-negative bacilli, 50 μl of the suspension was diluted in 15 ml of broth. The broth was inoculated onto Merlin MICRONAUT 384-well antimicrobial susceptibility testing plates for gram-positive bacteria (GP plates) and gram-negative bacteria (GN plates), respectively, designed for the German Network for Antimicrobial Resistance Surveillance (GENARS; www.genars.de), by using the automated Merlin Sprint device. For testing of the majority of antibiotics, the plates contained eight dilutions of the antibiotic for the determination of a real MIC. Breakpoint testing was done with fusidic acid and netilmicin on the GP plate and with aztreonam, cefotiam, mezlocillin, and netilmicin on the GN plate. Inoculated plates were incubated for 18 to 24 h at 36°C under ambient air. For the testing of streptococci, 200 μl of the suspension was diluted with 15 ml of Mueller-Hinton II broth (containing 0.25 g/liter phytagel and 200 μl of lysed horse blood). The broth was inoculated onto Merlin MICRONAUT 96-well testing plates for streptococci (Strep plates), and plates were incubated for 18 to 24 h at 36°C in a 5% CO_2 atmosphere. Reading of all plates was done with a photometer (Merlin) interpreting an optical density of >0.1 to indicate growth. Obtained MICs were interpreted with the advanced expert system (AES) MCN-6 of Merlin MICRONAUT by using the interpretation guidelines of the German Standardization Institute (Deutsches Institut für Normung) (7) and validated by a clinical microbiologist. A sheep blood agar was inoculated with suspensions from all McFarland standards used for susceptibility testing and incubated at 36°C for 18 to 24 h in order to control for growth, mixed cultures, and possible contamination.

Direct susceptibility testing. For direct testing, 8 ml of the positive blood culture medium was centrifuged at 130 × *g* (800 rpm) for 10 min. The supernatant was transferred into a new tube and centrifuged at 1,800 × g (3,000 rpm) for 5 min. The resultant pellet was diluted in sterile 0.9% saline to prepare a 0.5-McFarland-standard suspension, and the suspension was processed as described above. The antimicrobial resistance testing panel was chosen according to the results of the Gram staining of the positive blood cultures. For the testing of gram-positive cocci in clusters and gram-positive diplococci and cocci in short chains, suggestive of enterococci, the GP plate was used. If small gram-positive cocci in chains, suggestive of streptococci, were seen, the Strep plate was chosen. For testing of gram-negative bacilli, the GN plate was used.

Identification of bacterial strains. Identification of all bacterial species apart from most staphylococci was done by API immediately after obtaining pure subcultures (API 20 Strep, API Rapid ID 32 Strep, API 20 E, and API 20 NE; BioMérieux, Germany). For staphylococci, diagnosis was based on typical microscopy observations and morphology (color and hemolysis, etc.), positive catalase reactions, and growth on mannitol-salt agar. *Staphylococcus aureus* was differentiated from coagulase-negative staphylococci by morphology and the presence of the positive clumping factor (Slidex; BioMérieux). If differentiation was ambiguous, aurease detection by RAPIDEC Staph (BioMérieux) and an API 20 Staph was done. For all isolates for which biochemical identification was ambiguous ($n = 5$), sequencing of the complete 16S rRNA genes was performed as described previously (17, 1). All isolates included in the study were stored in Microbank tubes (Doenitz ProLab, Augsburg, Germany) at −20°C.

Confirmative susceptibility testing of staphylococci. Identification of the staphylococcal *mecA* gene by PCR was done as described previously (25). Quinupristin-dalfopristin (Synercid) testing by the E-test (Viva Diagnostika, Koeln, Germany) was done on Mueller-Hinton agar (Heipha, Heidelberg, Germany) using a 0.5-McFarland-standard suspension of the respective strain. Plates were incubated in ambient air at 36°C for 24 h.

Quality control. Quality control strains, including *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, and *vanA*-positive *Enterococcus faecium* (DSM 17050), were investigated daily (each strain three times a week) by the standard procedure. In addition, precision of the standard method was determined by assessing *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* ATCC 25922 in 10 replicates of a suspension corresponding to a single McFarland standard (data not shown). Differences exceeding a range of two twofold dilutions of the MIC were observed with imipenem, ertapenem, and tobramycin. Therefore, these antibiotics were not included in the data analysis. Precision of the direct AST method was determined by the investigation of 10 blood cultures containing blood from a healthy volunteer spiked with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Differences exceeding a range of two twofold dilutions of the MIC and results beyond the given limits of the German Standardization Institute (7) were observed only with imipenem.

Data analysis. For each antibiotic test result, raw MICs and validated interpretation results (susceptible, intermediate susceptible, resistant [SIR]) from direct and standard testing were compared after AES validation. MIC agreement was defined as results for a MIC pair in which the MIC from direct testing was within one twofold dilution of the MIC from standard testing (11). Category (SIR) agreement was defined as concordance between validated SIR. Test results with SIR discrepancies that did in fact display MIC agreement were also counted as having SIR agreement in order to minimize method-inherent artifacts, e.g., SIR discrepancies introduced by AES validation. Regarding breakpoint testing of antibiotics, results for only fusidic acid (GP plate) and aztreonam (GN plate) were included in the data analysis since artifacts in SIR validation introduced by the AES could be excluded for these antibiotics. A very major error was defined as a result of susceptibility in the direct testing and resistance in the standard testing, a major error was defined as a result of resistance in the direct testing and susceptibility in the standard testing, and a minor error was defined as all other discrepancies between results from direct and standard testing (11).

RESULTS

Study population. During the study period, direct AST was done with 850 positive blood cultures, including 637 cultures in aerobic bottles and 213 cultures in anaerobic bottles. Out of the 850 blood cultures, 146 were positive for gram-negative rods (17.2%), 562 showed gram-positive cocci in clusters (66.2%), 134 showed gram-positive diplococci and cocci in short chains (15.7%), and 8 showed small gram-positive cocci in chains, suggestive of streptococci (0.9%), in the initial Gram staining performed after positive signaling of the bottles in the BACTEC system.

Among all 850 blood cultures, direct AST of 702 samples (82.6%) could be evaluated. Susceptibility testing of 148 samples could not be evaluated due to the following reasons: detection of polymicrobial growth in the blood cultures in 69 samples (8.1%) after overnight incubation, failure of growth in 39 samples (4.6%) during the AST, selection of an incorrect direct AST panel due to ambiguous Gram staining results for 27 samples (3.2%), contamination of the direct AST plates for 3 samples (0.3%), growth of a bacterial species that was not suitable for AST with the methods used in this study in 9

samples (1.0%; organisms included six anaerobes, two isolates of *Lactococcus lactis*, and one isolate of *Moraxella catarrhalis*), and the inability to prepare the inoculum for direct AST due to extensive hemolysis by one isolate (0.1%) of *Enterococcus faecalis*.

Among blood cultures with polymicrobial growth in the direct AST ($n = 69$), mixtures mainly of different gram-positive species, predominantly coagulase-negative staphylococci and enterococci, were found. In 12 samples, gram-negative bacilli were involved in mixtures with gram-positive cocci or other gram-negative bacilli.

Blood cultures with failed growth in direct AST ($n = 39$) comprised the following species: coagulase-negative staphylococci ($n = 25$), *Staphylococcus aureus* ($n = 3$), *Micrococcus luteus* ($n = 2$), *Acinetobacter lwoffii* ($n = 1$), *Escherichia coli* ($n = 1$), *Gemella haemolysans* ($n = 1$), *Rothia mucilaginosa* ($n = 1$), *Streptococcus agalactiae* ($n = 1$), *Streptococcus anginosus* ($n = 1$), *Streptococcus mitis* ($n = 1$), *Streptococcus pneumoniae* ($n = 1$), and *Streptococcus sanguinis* ($n = 1$).

Incorrect direct AST panels were chosen for 27 samples, including 21 with isolates of *Streptococcus* spp. (including 10 isolates of *Streptococcus pneumoniae*) and 1 with an isolate of *Gemella haemolysans* that were tested on the GP plate (Gram staining results were suggestive of enterococci), 4 with isolates of *Enterococcus faecalis* that were tested on Strep plates (Gram staining results were suggestive of streptococci), and 1 with an isolate of *Acinetobacter lwoffii* that was misidentified as gram-positive cocci.

If isolates of the same species with identical antimicrobial susceptibility testing profiles were detected in more than one blood culture within 14 days, the direct AST of the first isolate only was repeated by the standard method and subsequent isolates ($n = 198$) were not included in the AST study. By this procedure, a total of 504 blood cultures from 409 patients were finally available for comparison of direct and standard AST methods.

Gram-negative bacilli. Direct and standard susceptibility testing was done on 110 isolates of gram-negative bacilli (Table 1). Twenty-four antibiotics were investigated, and 2,637 organism-antibiotic combinations were available for data analysis. MIC agreement was found for 98.1% of all combinations (Table 2). Category agreement (SIR agreement) was found for 99.0% (Table 2). Minor errors occurred in results for 0.8% of the combinations, major errors in results for 0.2%, and very major errors in results for 0.04% (Table 2). False susceptibility results from direct testing were noted only for piperacillin-tazobactam with one isolate of *Escherichia coli* and for aztreonam with one isolate of *Morganella morganii*. Altogether, the study population included six isolates of members of the *Enterobacteriaceae* with an AmpC–β-lactamase phenotype and 26 isolates of members of the *Enterobacteriaceae* resistant to amoxicillin-clavulanate.

Gram-positive cocci (GP plate). Direct and standard susceptibility testing was done with 394 isolates of gram-positive cocci (Table 1). Out of these 394 isolates, 373 isolates of staphylococci, enterococci, *Micrococcus luteus*, and *Kocuria* spp. were tested with the GP plate and 21 isolates of *Streptococcus* spp. were tested with the Strep plate. Concerning the GP plate, 24 antibiotics were investigated and 8,951 organism-antibiotic combinations were available for data analysis. Altogether, resistance against penicillin, oxacillin, and erythromycin in coagulase-negative staphylococci was noted for 251 (89%), 223 (79%), and 202 (72%) isolates, respectively, and 30 isolates of *Staphylococcus aureus* (65%) were resistant to penicillin. MIC agreement was found for 95.6% of all combinations (Table 3). SIR agreement was found for 98.8% (Table 3). Minor errors occurred in results for 0.5%, major errors in results for 0.4%, and very major errors in results for 0.3% (Table 3).

Regarding the important antibiotic oxacillin, discrepant results of direct and standard AST were noted for five isolates of coagulase-negative staphylococci (Table 3), including three isolates of *Staphylococcus epidermidis* and two of *Staphylococcus hominis*. In all five isolates, the presence of the *mecA* gene could be demonstrated by PCR. Therefore, three isolates (two *Staphylococcus hominis* and one *Staphylococcus epidermidis*) are correctly classified as having results with very major errors

TABLE 1. Species distribution among the positive blood cultures available for direct and standard antimicrobial susceptibility testing

Type of organisms	Isolate(s) (*n*)	Plate type used for susceptibility testing
Gram-negative bacilli	*Escherichia coli* (55)	GN
	Pseudomonas aeruginosa (16)	GN
	Klebsiella pneumoniae (12)	GN
	Enterobacter cloacae (7)	GN
	Klebsiella oxytoca (4)	GN
	Citrobacter freundii (2)	GN
	Stenotrophomonas maltophilia (2)	GN
	Acinetobacter baumannii (1)	GN
	Acinetobacter species (1)	GN
	Citrobacter koseri (1)	GN
	Citrobacter species (1)	GN
	Enterobacter aerogenes (1)	GN
	Enterobacter hormaechei (1)	GN
	Flavimonas oryzihabitans (1)	GN
	Morganella morganii (1)	GN
	Pantoea agglomerans (1)	GN
	Salmonella enterica serovar Typhi (1)	GN
	Serratia liquefaciens (1)	GN
	Serratia marcescens (1)	GN
Gram-positive cocci	Coagulase-negative staphylococci (281)	GP
	Staphylococcus aureus (44)	GP
	Methicillin-susceptible *Staphylococcus aureus* (40)	GP
	Methicillin-resistant *Staphylococcus aureus* (4)	GP
	Enterococcus faecium (24)	GP
	Vancomycin-resistant *Enterococcus faecium* (2)	GP
	Enterococcus faecalis (14)	GP
	Micrococcus luteus (7)	GP
	Enterococcus gallinarum (2)	GP
	Kocuria species (1)	GP
	Streptococcus mitis (9)	Strep
	Streptococcus anginosus (3)	Strep
	Streptococcus oralis (2)	Strep
	Streptococcus pneumoniae (2)	Strep
	Streptococcus sanguinis (2)	Strep
	Streptococcus agalactiae (1)	Strep
	Streptococcus dysgalactiae subsp. *equisimilis* (1)	Strep
	Streptococcus pyogenes (1)	Strep

TABLE 2. Correlation of results of direct and standard antimicrobial susceptibility testing of gram-negative bacilli (n = 110) by using the GN plate

Drug	No. of organism-drug combinations with results indicating:				
	MIC agreement	SIR agreement	Very major error	Major error	Minor error
Amikacin	106	107	0	0	3
Amoxicillin-clavulanate	108	110	0	0	0
Ampicillin	108	109	0	0	1
Ampicillin-sulbactam	107	108	0	0	2
Aztreonam	NA[a]	107	1	0	2
Cefaclor	109	110	0	1	0
Cefepime	108	108	0	0	1
Cefotaxime	109	110	0	0	0
Cefoxitine	108	108	0	0	1
Cefpodoxime	108	109	0	1	1
Cefpodoxime-clavulanate	109	110	0	0	0
Ceftazidime	109	110	0	0	0
Cefuroxime	109	110	0	0	0
Ciprofloxacin	109	109	0	0	0
Doxycyclin	109	109	0	1	0
Gentamicin	103	106	0	0	4
Levofloxacin	110	110	0	0	0
Meropenem	105	110	0	0	0
Moxifloxacin	109	109	0	0	1
Piperacillin	105	108	0	0	2
Piperacillin-sulbactam	106	108	0	0	2
Piperacillin-tazobactam	108	108	1	0	0
Trimethoprim	109	109	0	1	0
Trimethoprim-sulfamethoxazole	108	108	0	0	1
Total (%)	2,479 (98.1)	2,610 (99.0)	2 (0.08)	4 (0.2)	21 (0.8)

[a] NA, not applicable due to breakpoint testing.

for oxacillin. However, for the two isolates (both *Staphylococcus epidermidis*) classified as having results with major errors, the direct oxacillin testing gave the correct result.

We observed very major errors in results with quinupristin-dalfopristin for four isolates (Table 3), including two isolates of *Staphylococcus aureus* (one methicillin-resistant strain and one methicillin-susceptible strain) and two coagulase-negative staphylococci. Since the level of quinupristin-dalfopristin resistance is low in Germany, the observed resistance demonstrated in the standard AST was questioned and the AST was repeated with stored subcultures of all four isolates. Repeated standard AST revealed susceptibility to quinupristin-dalfopristin in all isolates. MICs were within one dilution of those found in the direct testing (MIC from direct testing, ≤0.5 μg/ml; MIC from initial standard testing, 2 to 4 μg/ml; MIC from repeated standard testing, ≤0.5 to 1 μg/ml). In addition, a quinupristin-dalfopristin E-test was done with all four isolates and this test confirmed susceptibility to quinupristin-dalfopristin (MIC, 0.38 to 0.75 μg/ml). Thus, the supposed very major errors were caused by the false detection of quinupristin-dalfopristin resistance in the initial standard testing.

Streptococci (Strep plate). For streptococci on the Strep plate, 12 antibiotics were tested and 231 organism-antibiotic combinations were available for data analysis. MIC agreement and SIR agreement were found for 96.5% and 97.8% of combinations, respectively. Minor errors occurred in results for 0.4% of combinations, major errors in results for 0%, and very major errors in results for 1.7% (Table 4). False susceptibility results from direct testing were noted for erythromycin and clindamycin with one isolate of *Streptococcus oralis* and for trimethoprim-sulfamethoxazole with one isolate each of *Streptococcus anginosus* and *Streptococcus pyogenes*. Altogether, resistance against erythromycin and clindamycin and penicillin was noted in nine (43%) and five (24%) isolates of streptococci, respectively.

After termination of the study, 12 further blood cultures growing streptococci (including five *Streptococcus mitis*, two *Streptococcus anginosus*, two *Streptococcus pneumoniae*, two *Streptococcus pyogenes*, and one *Streptococcus oralis* strain) were evaluated with both methods during clinical diagnostics. All 132 organism-antibiotic combinations revealed SIR agreement. Thus, for the whole population of 33 isolates, minor errors occurred in results for 0.3% of combinations, major errors in results for 0%, and very major errors in results for 1.1% (data not shown).

DISCUSSION

Shortening the time to results of antimicrobial susceptibility testing of blood culture isolates can lead to significant reductions in patient morbidity, mortality, and costs (3, 9, 27). Therefore, we evaluated the accuracy of the MICRONAUT system for direct AST of positive blood cultures under routine conditions in a clinical microbiology laboratory. The MICRONAUT system is a commercially available, automated, microtiter plate-based broth dilution AST system (2, 16). Altogether, 850 positive blood cultures were investigated on a daily basis including weekends during a period of 9 months. Five hundred four isolates and 11,819

TABLE 3. Correlation of results of direct and standard antimicrobial susceptibility testing of gram-positive cocci (n = 373) by using the GP plate

Drug	No. of organism-drug combinations with results indicating:				
	MIC agreement	SIR agreement	Very major error	Major error	Minor error
Amoxicillin-clavulanate	340	372	0	1	0
Ampicillin	322	372	0	1	0
Cefazolin	349	370	1	2	0
Cefuroxime-axetil	352	371	1	1	0
Ciprofloxacin	364	369	2	2	0
Clindamycin	365	371	1	1	0
Doxycyclin	361	366	0	1	6
Erythromycin	362	365	2	5	1
Fosfomycin	363	367	3	3	0
Fusidic acid	NA[a]	369	0	3	2
Gentamicin	353	364	1	3	5
Levofloxacin	364	367	1	1	4
Linezolide	357	373	0	0	0
Meropenem	348	370	1	2	0
Moxifloxacin	364	366	0	2	5
Mupirocin	372	372	1	0	0
Oxacillin	348	368	3	2	0
Penicillin	337	370	1	2	0
Quinupristin-dalfopristin	366	367	4	0	0
Rifampin	368	368	0	2	3
Teicoplanin	347	356	0	0	17
Telithromycin	359	370	0	3	0
Trimethoprim-sulfamethoxazole	355	366	1	1	5
Vancomycin	368	373	0	0	0
Total (%)	8,553 (95.6)	8,841 (98.8)	23 (0.3)	38 (0.4)	48 (0.5)

[a] NA, not applicable due to breakpoint testing.

organism-antibiotic combinations could be evaluated for comparison of both direct and standard AST methods. Thus, the number of isolates included in this study exceeds by far those included in previously published studies of direct AST with positive blood cultures (4–6, 12, 14, 18, 21, 24, 26, 28).

The overall MIC agreement between results from direct and standard susceptibility testing of gram-negative and gram-positive isolates was high (95.6% to 98.1%) (Table 2 to Table 4). For every antimicrobial agent except ampicillin on the GP plate, the MIC agreement was >90%, as required by the selection criteria for an antimicrobial susceptibility testing system proposed by Jorgensen (19). Categorical error rates were very low and did not exceed the limits proposed by Jorgensen (19), i.e., very major errors occurred in less than 1.5% of results for all species investigated and the overall percentage of errors attributable to the new procedure did not exceed 5%.

For gram-negative isolates, the very major error rate was as low as 0.08%. Very major errors were seen only with aztreonam and piperacillin-tazobactam for two members of the *Enterobacteriaceae*. Concerning these antibiotics, very major errors in results from direct AST of gram-negative bacilli were also detected in recent studies using the MicroScan (28), Phoenix (12), and

TABLE 4. Correlation of results of direct and standard antimicrobial susceptibility testing of streptococci (n = 21) by using the Strep plate

Drug	No. of organism-drug combinations with results indicating:				
	MIC agreement	SIR agreement	Very major error	Major error	Minor error
Amoxicillin-clavulanate	20	21	0	0	0
Ampicillin	21	21	0	0	0
Ceftriaxone	21	21	0	0	0
Cefuroxime	21	21	0	0	0
Ciprofloxacin	20	20	0	0	1
Clarithromycin	19	20	1	0	0
Clindamycin	20	21	0	0	0
Doxycyclin	20	21	0	0	0
Erythromycin	20	20	1	0	0
Penicillin	21	21	0	0	0
Trimethoprim-sulfamethoxazole	19	19	2	0	0
Total (%)	223 (96.5)	226 (97.8)	4 (1.7)	0 (0)	1 (0.4)

VITEK 2 (6, 21) systems. However, very major errors involving the expanded- and broad-spectrum cephalosporins, for example, cefotaxime, cefuroxime, and ceftazidime, as frequently observed with other automated systems (4, 6, 12, 21, 28), were not detected in our study. Due to the observed very low rate of errors, direct results obtained with the MICRONAUT system are sufficiently reliable to be reported to the clinician.

Concerning gram-positive species, only isolates tested on the GP plate (mainly staphylococci and enterococci) should be evaluated since the number of streptococci investigated on the Strep plate within this study ($n = 21$) is too small for further analysis. After termination of the study, however, 12 additional blood cultures growing streptococci were investigated and did not show any errors in direct AST. Nevertheless, since only a very small number of resistant streptococci (4/21 penicillin resistant and 9/21 erythromycin resistant) and no penicillin-resistant pneumococci were included in the study, no reliable statement can be made regarding the occurrence of very major errors for streptococci.

A high rate of very major errors was observed with direct testing of quinupristin-dalfopristin with gram-positive cocci on the GP plate (Table 3). These very major errors could, however, be disproved by repeated testing and were most probably caused by incorrect automated reading of the plate, such as that from humidity-generated condensation. Three very major and two major errors were detected with oxacillin for five isolates of coagulase-negative staphylococci. Interestingly, the *mecA* gene was present in all five isolates, confirming the very major errors but disproving the major errors. The latter phenomenon may be explained by a heterogenic resistance pattern, the presence of both oxacillin-susceptible and oxacillin-resistant subpopulations of the respective isolate in the blood culture bottle, and the predominant growth of the susceptible population in the subculture and the standard AST. In one case, the bottle with the positive blood culture was still available when the presumptive major error was observed. Further subcultures from the blood culture bottle confirmed our assumption, showing a mixed population of oxacillin-susceptible and -resistant colonies. Altogether, antibiotics corresponding to detectable very major errors in our study included mainly those antibiotics for which errors with the VITEK 2 system were described previously (6, 8). A too-low inoculum or slow growth of the bacteria probably caused the discrepant results. Concerning minor errors, a high number was seen with teicoplanin. These errors were seen exclusively with coagulase-negative staphylococci, included equal numbers of falsely high and falsely low MICs, and may probably be explained by the lower precision of the method for measurement of this antibiotic due to antibiotic- and/or system-inherent reasons.

A critical technical step in direct AST with positive blood cultures is the preparation of the inoculum (10, 22). Blood cells, cellular debris, and constituents of the blood culture medium, etc., may hamper the preparation of a defined-McFarland-standard suspension and may disturb the testing procedure since the bacteria are often present in low concentrations in the positive blood culture medium. Enrichment with bacterial cells for direct AST by using serum separator tubes (BD) has been evaluated recently (6, 12). In our study, we developed a simple two-step centrifugation method for the separation of bacterial cells from positive blood cultures. Apart from that for one blood culture growing hemolytic *Enterococcus faecalis*, the inoculum for direct AST, i.e., the 0.5-McFarland-standard suspensions, could be prepared easily within 20 min and was macroscopically devoid of red blood cells. The density of bacterial growth observed on the direct AST quality control plates did not differ from that on standard quality control plates, and the results for the quality control strains investigated by the direct AST method were within the given limits. Furthermore, repeated direct testing of single strains from individual patients revealed a high rate of agreement (data not shown). Thus, this preparation method is reliable and approximately as fast as but much cheaper than the serum separator tube method.

Polymicrobial growth in direct AST was observed in 8.1% of blood cultures, which is slightly higher than in other studies (4, 28). Blood samples were taken by both venipuncture and line draw by medical personnel of the respective wards. Due to the absence of a specialized blood collection team, a higher rate of contamination in this study than in previous studies may be assumed. Also, a much higher number of gram-positive isolates was included in this study than in the above-mentioned studies and the majority of polymicrobial cultures included mixtures of different gram-positive cocci.

An important task in direct AST of gram-positive cocci in chains was to choose the correct test panel, i.e., the GP plate for enterococci or the Strep plate for streptococci. For most samples, the Gram staining result allowed the selection of the correct plate; however, microscopic misidentification of streptococci, especially *Streptococcus pneumoniae*, as enterococci was a problem and led to the delay of AST of 21 isolates. Nevertheless, in the majority of clinical microbiology laboratories, direct AST of streptococci is not even available.

In conclusion, direct AST of bacterial isolates from positive blood cultures with the Merlin MICRONAUT system is a reliable technique that can reduce the time to results of blood culture testing by omitting repeat testing from subcultures and facilitate earlier initiation of pathogen-directed antimicrobial therapy in septic patients. Thereby, it may have a positive impact on patient care (3, 9, 27), allow an earlier switch from a broad-spectrum antimicrobial to a more appropriate pathogen-adapted antibiotic, and thus prevent the development of resistance. Furthermore, reliable direct AST may facilitate the reduction of the overall consumption of antibiotics and health care costs. The method is suitable for both gram-negative bacilli and gram-positive cocci and is robust enough to be used on a 7-days-a-week basis in a routine clinical microbiology laboratory. In contrast to the commonly used VITEK (BioMérieux) and BD Phoenix (BD) systems, the Merlin MICRONAUT system offers the advantages of a broader panel of antibiotics on one test plate, the determination of definitive MICs of the majority of antibiotics, and visual control of bacterial growth on the plates. For the first time, direct AST of streptococci was evaluated in this study, with promising results.

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