Strategy for Rapid Identification and Antibiotic Susceptibility Testing of Gram-Negative Bacteria Directly Recovered from Positive Blood Cultures Using the Bruker MALDI Biotyper and the BD Phoenix System

Jana L. Wimmer,^a S. Wesley Long,^{a,b} Patricia Cernoch,^a Geoffrey A. Land,^a James R. Davis,^a James M. Musser,^{a,b} and Randall J. Olsen^{a,b}

Department of Pathology and Genomic Medicine, The Methodist Hospital,^a and Center for Molecular and Translational Human Infectious Disease Research, The Methodist Hospital Research Institute,^b Houston, Texas, USA

Decreasing the time to species identification and antibiotic susceptibility determination of strains recovered from patients with bacteremia significantly decreases morbidity and mortality. Herein, we validated a method to identify Gram-negative bacteria directly from positive blood culture medium using the Bruker MALDI Biotyper and to rapidly perform susceptibility testing using the BD Phoenix.

Bloodstream infections caused by Gram-negative bacteria are a leading cause of morbidity and mortality among hospitalized patients (12, 14). Inasmuch as decreasing the time to identify the causative organism and determine its antibiotic susceptibility can significantly improve outcomes in patients with bacteremia or sepsis (1, 7, 16), we validated a method to rapidly identify and perform susceptibility testing for Gram-negative bacteria directly recovered from positive blood culture medium. Our strategy used matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper; Bruker Daltonics, Billerica, MA) and automated antibiotic susceptibility testing (Phoenix, BD, Sparks, MD) instruments.

The study was performed in two parts. First, we created a training test set of 50 simulated bacteremia specimens. The 10 Gram-negative species included in the training set were distributed in a ratio that is representative of the bacteremia specimens processed in our clinical microbiology laboratory. Organisms were selected from cryopreserved stocks of strains purchased from the American Type Culture Collection (ATCC, Manassas, VA), strains recovered from College of American Pathologists (CAP, Northfield, IL) proficiency testing surveys, and patient isolates that were collected in accordance with a protocol approved by the institutional review board at The Methodist Hospital. Each simulated bacteremia specimen was prepared at a clinically relevant concentration ($\sim 10^3$ to 10^5 CFU/ml) using known Gram-negative strains (Table 1). Briefly, each strain was grown overnight on tryptic soy agar supplemented with 5% sheep blood (BD), colonies were transferred to 5 ml sterile saline using an inoculating loop, and the suspension was vortexed for 30 s. The concentration of bacteria in each suspension was estimated using a nephelometer (Phoenix-Spec, BD) and McFarland equivalence turbidity standards (Remel, Lenexa, KS). A 10-ml simulated sample was then prepared using an appropriate dilution factor, inoculated into an aerobic blood culture bottle (Bactec Standard 10 Aerobic F Medium; BD), and incubated in an automated instrument (Bactec FX; BD) according to the manufacturer's instructions. Second, we created a validation test set of 60 positive patient blood cultures in which the initial Gram stain indicated a Gram-negative organism. Polymicrobial cultures were excluded from the study. The organism

identification and susceptibility testing were performed by blinded laboratory personnel.

After being flagged as positive for growth by the blood culture instrument, 6 ml of broth was transferred from the blood culture bottle with a syringe to an 8.5-ml serum separator tube (Vacutainer SST Plus; BD) and centrifuged at 2,000 rpm for 15 min at room temperature. The supernatant was aspirated, using caution to not disrupt the loosely formed pellet of bacteria present at the surface of the polymeric gel. The MALDI-TOF mass spectrometry identification and antibiotic susceptibility panels were then prepared simultaneously. For rapid organism identification, an aliquot of the pelleted bacteria was collected by gently touching it with a cotton swab (Pur-Wraps; Puritan Medical Products Company, Guilford, ME) and spotted onto the stainless steel target. Importantly, in evaluating several different swabs, we determined that a loosely wound, coarsely textured cotton tip provided the most consistent transfer of bacteria to the target. The bacteria were then allowed to dry at room temperature, overlaid with 1 μ l of α -cyano-4-hydroxycinnamic acid (HCCA) matrix, and analyzed using the MALDI-TOF mass spectrometer (MALDI Biotyper with FlexControl software; Bruker Daltonics) according to the manufacturer's instructions. Since MALDI-TOF mass spectrometry cannot reliably distinguish between *Escherichia coli* and *Shigella* spp., a spot indole test was performed to confirm the identification. Each unknown organism was also identified using conventional biochemical phenotyping methods (Phoenix; BD) on colonies isolated from subcultures of the positive blood culture medium. Any discordant results from the two identification methods were resolved by 16S rRNA gene sequencing (ARUP Laboratories, Salt Lake City, UT). For rapid antibiotic suscep-

Received 11 February 2012 Returned for modification 12 March 2012

Accepted 5 April 2012

Published ahead of print 18 April 2012

Address correspondence to Randall J. Olsen, RJolsen@tmhs.org.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.00409-12

TABLE 1 Organism identification and antibiotic susceptibility testing results

Gram-negative strain	No. of concordant identifications/ total no. of identifications (%)		No. of organism-antibiotic tests	No. of each type of error in antibiotic susceptibility testing (% of total no. of tests)		
	Training set	Validation set		Very major	Major	Minor
<i>Achromobacter</i> species		1/1	13 ^a	0	0	2
<i>Citrobacter freundii</i>	2/2	1/1	57	0	0	2
<i>E. cloacae</i> complex	4/5	1/1	114	0	0	2
<i>Enterobacter aerogenes</i>		2/2	38	0	0	0
<i>E. coli</i> ^b	15/15	31/31	854	0	4 ^g	7
<i>Klebsiella pneumoniae</i> ^c	8/8	16/16	442	1 ^d	0	2
<i>Morganella morganii</i>	2/2	1/1	54	0	0	3
<i>Proteus mirabilis</i>	5/5	2/2	126	3 ^e	0	0
<i>Providencia stuartii</i>	1/1		19	0	0	0
<i>Pseudomonas aeruginosa</i>	6/6	2/2	80	1 ^f	2 ^h	7
<i>Serratia marcescens</i>	3/3	2/2	90	0	0	0
<i>S. maltophilia</i>	2/3	1/1	8	0	0	1
Total	48/50 (96)	60/60 (100)	1,882	5 (0.26)	6 (0.32)	26 (1.37)

^a For all organisms, analysis of the antibiotic susceptibility test data of the direct testing method compared to those of the conventional method was limited to the agents reported for each strain.

^b Ten *E. coli* strains were extended-spectrum β -lactamase (ESBL)-producing organisms.

^c Seven *K. pneumoniae* strains were ESBL-producing organisms.

^d The discordant agent was tetracycline.

^e The discordant agents were cefazolin in 1 strain and cefazolin and cefuroxime in 1 strain.

^f The discordant agent was aztreonam.

^g The discordant agents were ampicillin in 1 strain, tetracycline in 1 strain, and trimethoprim-sulfamethoxazole and cefepime in 1 strain.

^h The discordant agents were imipenem in 1 strain and piperacillin-tazobactam in 1 strain.

tibility testing, an aliquot of the pelleted bacteria was resuspended (Phoenix ID broth; BD), processed using the autoincubator (Phoenix AP; BD), and analyzed on an automated instrument (Phoenix; BD) according to the manufacturer's instructions. The MICs of 2 to 20 antimicrobial agents, selected as appropriate for each organism (Table 2), were determined for each strain. Antibiotic susceptibility results using the direct pellet method were compared to those generated by the conventional method (Phoenix; BD) that tests colonies isolated from subcultures of the positive blood culture medium. Any strain-antibiotic combination demonstrating a >1 -fold dilution difference in the MIC or a change in the categorical drug-susceptible/resistant interpretation between the two methods was resolved by performing an epsilometer test (bioMérieux, Durham, NC). Categorical disagreements were scored as very major errors (susceptible by the direct method and resistant by the reference method), major errors (resistant by the direct method and susceptible by the reference method), or minor errors (intermediate by one method and susceptible or resistant by the other method) as previously described (6).

Results of the rapid identification study using the MALDI-TOF mass spectrometer were highly concordant with those based on conventional biochemical phenotypes and 16S rRNA gene sequences (108/110 identifications were concordant [98%]) (Table 1). Both discordant identifications occurred in the training set. The MALDI-TOF mass spectrometer did not generate a reliable identification score for one *Enterobacter cloacae* complex strain, and it identified one *Stenotrophomonas maltophilia* strain to only the genus (Table 1). A similar level of diagnostic accuracy for identifying Gram-negative bacteria directly from positive blood culture bottles has been previously reported (4, 11, 15). Results of the rapid susceptibility study using pelleted bacteria directly recovered from the positive blood culture medium were also highly

concordant with those of the conventional method using colonies isolated by subculture (Table 1). A similar level of accuracy for susceptibility testing directly from positive blood culture bottles has been previously reported (3, 5, 8–10). The training set and validation set included totals of 821 and 1,074 organism-antibiotic combinations, respectively. In total, 5 very major errors (0.26%), 6 major errors (0.32%), and 26 minor errors (1.37%) were recorded. These data are consistent with accepted error rates for clinical microbiology laboratories (2, 13). No significant trend in the errors was observed for any organism or antibiotic agent tested (chi-square test, Prism 4; GraphPad Software, La Jolla, CA). Importantly, by bypassing the time-consuming subculture steps included in the conventional method, results from the direct testing method were generated a full day earlier (mean, 24.06 h; standard error of the mean [SEM], 0.84 h; range, 9.57 to 35.07 h).

In summary, the data demonstrate that Gram-negative organisms recovered from positive blood cultures can be accurately identified and rapidly assessed for antibiotic susceptibility directly from the primary broth. Although other investigators have also recently reported the successful identification of bacteria directly from positive blood culture medium using MALDI-TOF mass spectrometry (4, 11, 15), we are the first to couple rapid identification with rapid antibiotic susceptibility testing. To have a maximum benefit to patient care, particularly in Gram-negative sepsis, appropriate antibiotic therapy must be administered as soon as possible (12, 14). To this end, our strategy markedly reduced turnaround times for species identification and susceptibility testing. Due to operational constraints in our laboratory, specimens are processed in batches at predetermined time points. However, if the workflow were modified to immediately process positive blood cultures for Gram stain interpretation, MALDI-TOF analysis, and susceptibility testing when flagged by the instrument, clinically actionable results could be generated even more quickly.

TABLE 2 Organism-antibiotic susceptibility testing algorithm

Organism	Drug used for susceptibility testing																		
	Amikacin	Ampicillin	Ampicillin-sulbactam	Aztreonam	Cefazolin	Cefepime	Cefotaxime	Cefoxitin ^{a,c}	Ceftazidime	Ceftioxone	Cefuroxime	Ciprofloxacin	Gentamicin	Imipenem ^{b,c}	Levofloxacin	Meropenem ^{b,c}	Piperacillin-tazobactam ^{a,c}	Tetracycline	Tobramycin
<i>Achromobacter</i> species	X			X		X	X		X	X		X	X	X	X	X	X	X	X
<i>C. freundii</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>E. cloacae</i> complex	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>E. aerogenes</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>E. coli</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>K. pneumoniae</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>M. morgani</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
<i>P. mirabilis</i>	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>P. stuartii</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>P. aeruginosa</i>	X			X		X			X			X	X	X	X	X	X	X	X
<i>S. marcescens</i>	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
<i>S. maltophilia</i>															X				X

^a Antibiotic susceptibility test results for cefoxitin and piperacillin-tazobactam were suppressed in the microbiology report for extended-spectrum β -lactamase (ESBL)-producing organisms.

^b Imipenem and meropenem were tested for the organisms as indicated, but only one carbapenem agent was released in the microbiology report per the formulary of the hospital from which the specimen originated.

^c Analysis of the antibiotic susceptibility test data of the direct testing method compared to those of the conventional method was based on the agents reported for each strain.

The direct testing method described herein has been successfully implemented in our clinical microbiology laboratory that serves as the reference laboratory for The Methodist Hospital System. Validation studies are now under way to expand the strategy of rapid identification and antibiotic susceptibility testing to bacteremia caused by Gram-positive bacteria and yeast.

ACKNOWLEDGMENTS

We thank the clinical microbiology laboratory staff for assistance with the study and K. Stockbauer and P. Randall for assistance with manuscript preparation.

REFERENCES

- Barenfanger J, Drake C, Kacich G. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J. Clin. Microbiol.* 37:1415–1418.
- Baron EJ, et al. 2005. Cumitech 1C: blood cultures IV. ASM Press, Washington, DC.
- Bruins MJ, Bloembergen P, Ruijs GJ, Wolfhagen MJ. 2004. Identification and susceptibility testing of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by direct inoculation from positive BACTEC blood culture bottles into Vitek 2. *J. Clin. Microbiol.* 42:7–11.
- Buchan BW, Riebe KM, Ledebor NA. 2012. Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. *J. Clin. Microbiol.* 50:346–352.
- Chen JR, Lee SY, Yang BH, Lu JJ. 2008. Rapid identification and susceptibility testing using the VITEK 2 system using culture fluids from positive BacT/ALERT blood cultures. *J. Microbiol. Immunol. Infect.* 41:259–264.
- CLSI. 2008. Development of in vitro susceptibility testing criteria and quality control parameters, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA.
- Doern GV, Vautour R, Gaudet M, Levy B. 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *J. Clin. Microbiol.* 32:1757–1762.
- Funke G, Funke-Kissling P. 2004. Use of the BD PHOENIX Automated Microbiology System for direct identification and susceptibility testing of Gram-negative rods from positive blood cultures in a three-phase trial. *J. Clin. Microbiol.* 42:1466–1470.
- Gherardi G, et al. 2012. Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. *Diagn. Microbiol. Infect. Dis.* 72:20–31.
- Lupetti A, Barnini S, Castagna B, Nibbering PH, Campa M. 2010. Rapid identification and antimicrobial susceptibility testing of Gram-positive cocci in blood cultures by direct inoculation into the BD Phoenix system. *Clin. Microbiol. Infect.* 16:986–991.
- Moussaoui W, et al. 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of bacteria directly from blood culture vials. *Clin. Microbiol. Infect.* 16:1631–1638.
- Peleg AY, Hooper DC. 2010. Hospital-acquired infections due to Gram-negative bacteria. *N. Engl. J. Med.* 362:1804–1813.
- Rittenhouse SF, Miller LA, Utrup LJ, Poupard JA. 1996. Evaluation of 500 Gram-negative isolates to determine the number of major susceptibility interpretation discrepancies between the Vitek and MicroScan Walkaway for 9 antimicrobial agents. *Diagn. Microbiol. Infect. Dis.* 26:1–6.
- Russell JA. 2006. Management of sepsis. *N. Engl. J. Med.* 355:1699–1713.
- Stevenson LG, Drake SK, Murray PR. 2010. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J. Clin. Microbiol.* 48:444–447.
- Trenholme GM, et al. 1989. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. *J. Clin. Microbiol.* 27:1342–1345.