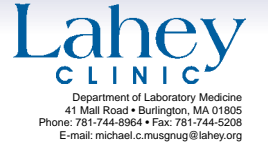


Direct Antimicrobial Susceptibility Testing of Blood Cultures by Sensititre® Broth Microdilution Plates

K. C. Chapin and M. C. Musgnug



ABSTRACT

Direct susceptibility reporting would benefit bacteremic patients by providing results more quickly. This study developed an algorithm for direct susceptibility testing from positive ESP™ (TREK Diagnostic Systems, Westlake, OH) blood culture broth into Sensititre® (TREK Diagnostic Systems, Westlake, OH) microdilution plates and evaluated accuracy compared to traditional methods. The following practical exclusion criteria were applied to gram positive (GP) organisms to avoid reporting susceptibilities for probable contaminants. A 2-hour tube coagulase (TC) test was performed to identify *S. aureus*. If the TC was positive, the GP organism was tested directly. If the TC was negative, then >1 set of bottles and/or a catheter culture had to be positive to warrant direct susceptibility testing. All gram negative (GN) organisms were tested. An aliquot of the blood culture was centrifuged and the resulting pellet was used to make a 0.5 McFarland suspension. Microdilution trays were inoculated and interpreted according to manufacturer's instructions. Viable counts were performed to ensure proper colony density was achieved. Results were compared to panels set up using colonies isolated 24 hours later from solid medium. A total of 199 isolates (81 patient isolates, 118 seeded) were evaluated for both essential (+/- one well) and categorical (sensitive, intermediate or resistant) agreement. GP organisms (n=93, 1214 drug/organism combinations) showed 98% essential agreement and error rates of 0.3% minor errors, no major (false resistance) errors, and 1.7% very major (false susceptibility) errors. For 106 GN organisms (1828 drug/organism combinations) essential agreement was 99%. Error rates were 0.5, 0, and 2.0% for minor, major and very major errors respectively. Performance was comparable for both GP and GN organisms and for both aerobic and anaerobic blood culture bottles. Using this direct testing methodology, reliable susceptibility results can be reported to physicians more quickly and result in appropriate modification of antibiotic therapy.

MATERIALS & METHODS

- Positive ESP blood cultures with Gram stains appearing unimicrobial were tested.
- The following exclusion criteria were applied to gram positive organisms to avoid testing CoNS that were probable contaminants:
 - Direct tube coagulase test was performed and read at 4 hr
 - If positive (SAUR), direct testing was deemed appropriate.
 - If negative (CoNS), >1 set of blood cultures and/or a catheter tip culture must be positive in order to warrant direct testing.
- Direct testing:**
 - A 10 ml aliquot of the blood culture broth was added to a Vacutainer® serum separator tube (SST™) (BD, Franklin Lakes, NJ)
 - The tube was centrifuged at 3500 rpm X 10 min.
 - The supernatant was decanted.
 - The pellet formed was used to prepare a suspension equivalent to 0.5 McFarland (1.5 X 10⁸ CFU/ml).
 - Colony counts were performed from the final inoculum to ensure the proper colony density (5 X 10⁷ CFU/ml) was achieved.

MATERIALS & METHODS con't

- Sensititre® MG (Gram positive) and MH (Gram negative) standard plates were inoculated and incubated according to manufacturer's instructions.
- Conventional testing:**
 - The positive blood culture was subcultured onto a blood agar plate (BBL, Sparks, MD) and incubated overnight at 35°C.
 - Isolated colonies were used to prepare a 0.5 McFarland suspension, which was then used to inoculate the MG and MH plates according to manufacturer's instructions.
- Plates were read at 18-24 hr both by Automated Reading and Incubation System (ARIS®) and manually with the SensiTouch®.
- Direct and conventional results were compared for essential agreement (+/- one well) and categorical agreement (S, I, R as per NCCLS).

Sensititre Standard Gram Positive (MG Plate) Antimicrobials

Antimicrobial	Range (µg/ml)	Antimicrobial	Range (µg/ml)
Ampicillin	0.12-8	Lomefloxacin	0.5-4
Ampicillin/Sulbactam	8/4-16/8	Nitrofurantoin	32-64
Cefazolin	2-16	Norfloxacin	4-8
Cephalothin	2-16	Oxofloxacin	0.5-4
Chloramphenicol	4-16	Oxacillin	0.25-2
Ciprofloxacin	0.06-2	Penicillin	0.03-8
Clarithromycin	0.12-4	Rifampin	0.5-2
Clindamycin	0.25-2	Tetracycline	0.25-8
Erythromycin	0.12-4	Trimethoprim/Sulfamethoxazole	0.5/9.5-2/38
Gentamicin	0.25-8	Vancocycin	0.5-16

Sensititre Standard Gram Negative (MH Plate) Antimicrobials

Antimicrobial	Range (µg/ml)	Antimicrobial	Range (µg/ml)
Amikacin	1-32	Ciprofloxacin	0.06-2
Amoxicillin/Clavulanic acid	0.5/0.25-16/8	Gentamicin	0.25-8
Ampicillin	0.5-16	Imipenem	0.25-8
Ampicillin/Sulbactam	8/4-16/8	Mezlocillin	4-64
Cefepime	0.25-16	Piperacillin	4-64
Cefotaxime	4-32	Piperacillin/Tazobactam	2/4-64/4
Ceftazidime	1-16	Ticarcillin/Clavulanic acid	4/2-64/2
Cefuroxime	1-16	Tobramycin	1-8
Cephalothin	1-16	Trimethoprim/Sulfamethoxazole	0.5/9.5-2/38

Isolates Tested Directly from Positive Blood Cultures

Organism	No. tested	Organism	No. tested
Gram-negative:			
<i>Escherichia coli</i>	23	<i>S. aureus</i> - methicillin sensitive	28
<i>Pseudomonas aeruginosa</i>	20	<i>Enterococcus faecalis</i>	24
<i>Klebsiella pneumoniae</i>	17	<i>Enterococcus faecium</i>	19
<i>Enterobacter cloacae</i>	15	<i>S. aureus</i> - methicillin resistant	14
<i>Klebsiella oxytoca</i>	8	Coagulase-negative <i>Staphylococcus</i>	5
<i>Acinetobacter</i> spp.	7	Other gram-positive organisms	3
<i>Enterobacter aerogenes</i>	6	Total Gram-positive	93
<i>Citrobacter</i> spp.	4		
<i>Proteus</i> spp.	3		
Other gram-negative organisms	3		
Total Gram-negative	106		

Total Isolates Tested^a 199

^a 81 patient bottles and 118 seeded bottles

Comparison of Susceptibility Results from Direct and Subcultured Positive Blood Culture Isolates^a

Organism	No. tested	Error Rates			
		Essential agreement ^b	Minor ^c	Major ^d	Very Major ^e
Gram-negative	106	99.0%	0.5%	0	2.0%
Gram-positive	93	98.0%	0.3%	0	1.7%
Total	199	98.6%	0.4%	0	1.9%

^a Sensititre MG and MH plates used for both direct and subcultured isolates

^b Results within one (1) two-fold dilution

^c One method: intermediate; One method: sensitive or resistant

^d False resistance

^e False susceptibility

Direct Testing Isolates Yielding Very Major Errors^a

Organism	Antimicrobial	No. (%) VM ^b Errors
<i>Staphylococcus aureus</i>	cefazolin	5 (12%)
<i>Staphylococcus aureus</i>	clarithromycin	1 (2%)
<i>Escherichia coli</i>	mezlocillin	3 (13%)
<i>Escherichia coli</i>	piperacillin	2 (9%)
<i>Klebsiella pneumoniae</i>	ampicillin	1 (6%)

^a No major errors were observed.

^b Very Major



CONCLUSIONS

- Direct susceptibility testing of positive ESP blood cultures via Sensititre broth microdilution plates performed well compared to conventional testing.
- Direct testing worked equally well for aerobic/anaerobic broths, gram positive/gram negative organisms, and patient/seeded isolates.
- Preliminary results show CoNS susceptibility performance differed between clinically significant and non-significant organisms. Inclusion criteria increased accuracy of direct susceptibility results.
- Enterococci* and coagulase-negative *Staphylococcus* must be read manually as per the package insert. TREK is currently seeking FDA approval to remove this limitation.
- Direct susceptibility results can help clinicians adjust antimicrobial therapy sooner to avoid extended use of broad-spectrum agents.
- Direct susceptibility testing could lead to improved outcomes for patients and reduced overall costs.

REFERENCES

- Barenfanger, J., et al. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J. Clin. Microbiol.* 37:1415-1418.
- Doern, G., et al. 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *J. Clin. Microbiol.* 32:1757-1762.

Presented at 102nd General Meeting, American Society for Microbiology Salt Lake City, UT, May 19, 2002.