P2811 TUESDAY

Evaluation of Rapid Antimicrobial Susceptibility Testing Methods for Gram-Negative Isolates

Amira Bhalodi
abhalodi@axdx.com

A. Bhalodi, S. Miquirray, I. Perez, J. Towne, R. Humphries
Accelerate Diagnostics Inc., Tucson, AZ

ACCELERATE DIAGNOSTICS™

MODIFIED ABSTRACT

Background: Traditional antimicrobial susceptibility testing (AST) can take 48-72 hours before yielding a result which can lead to detrimental outcomes in infected patients. Recently, more rapid methods have been introduced such as the automated Accelerate Pheno™ system (AXDX) as well as modified standard methods like direct disk diffusion. Herein we compare the performance of two rapid AST methods.
Materials/methods: AST was performed on 42 *Enterobacteriaceae* isolates with the following methods: broth microdilution (BMD), direct disk diffusion with reading performed 8 hours (dDD8), and AXDX. The dDD8 and AXDX methods were compared to BMD as the reference method. For dDD8 and AXDX, positive blood culture broth was utilized as inoculum. Categorical agreement (CA) was evaluated using the recommended 2018 CLSI *Enterobacteriaceae* breakpoints. Very major errors (VME) were defined as false susceptible isolates by the test method compared with the reference method of BMD. Major errors (ME) were defined as false resistance isolates. Minor errors (mE) were defined as one result in the intermediate category with the other in either susceptible or resistant category. The antimicrobial agents tested were: amikacin, ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, meropenem, piperacillin/tazobactam, sulfamethoxazole/trimethoprim, tobramycin.

Results: Overall CA and error rates are displayed in the table for both methods tested compared to BMD.

Test Method Compared to BMD	CA (%)	mE (%)	ME (%)	VME (%)
Direct Disk Diffusion 8 hour	92.22	5.56	1.03	4.50
Accelerate Pheno™ system	94.76	3.65	1.03	2.70

Conclusions: Both methods evaluated had CA above 90%. AXDX provides an accurate, automated and rapid identification and susceptibility result directly from a positive blood culture in approximately 7 hours. While the CA for direct disk diffusion was above 90%, the VME rate was above the 3% cut-off established by ISO/EUCAST guidance. Direct disk diffusion is more labor-intensive and requires use of a rapid identification method. In this data set, direct disk diffusion resulted in inter-operator variability between two independent reads 16.8% of the time.

BACKGROUND

Importance of early appropriate antimicrobial therapy for patients with suspected bloodstream infections is well established.[1] Until recently, FDA approved methods for susceptibility testing required the preparation of an inoculum from an isolate, thus requiring sufficient incubation time to obtain a pure culture. Various methods of direct susceptibility testing, such as direct disk diffusion and new technologies are being explored and implemented by clinical laboratories in an effort to provide AST results sooner. The Accelerate Pheno™ system is an FDA cleared device that provides identification and antimicrobial susceptibility in approximately 7 hours of a positive blood culture. The rapid disk diffusion method utilizes a positive blood culture in lieu of an inoculum generated from pure culture.[2]
The objective of this study was to compare the performance of two rapid AST methods, direct disk diffusion and the Accelerate Pheno™ system as compared to the reference method of BMD.

METHODS

42 *Enterobacteriaceae* isolates were tested (21 *Escherichia coli,* 12 *Klebsiella pneumoniae* 2 *Klebsiella oxytoca,* 3 *Citrobacter freundii,* 3 *Proteus mirabilis,* 1 *Enterobacter cloacae)*
Antimicrobial agents tested with the following disk content: amikacin 30 µg, ampicillin 10 µg, ampicillin/sulbactam 10/10 µg, aztreonam 30 µg, cefazolin 30 µg, cefepime 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, ciprofloxacin 5 µg, ertapenem 10 µg, gentamicin 10 µg, meropenem 10 µg, piperacillin/tazobactam 100/10 µg, sulfamethoxazole/trimethoprim 23.75/1.25 µg, tobramycin 10 µg
Direct Disk Diffusion Method: 4 drops of positive blood culture broth from a sub/venting unit were placed on a 150 mm Mueller Hinton agar (MHA) plate and swabbed to create a bacterial lawn. Antimicrobial susceptibility disks were placed on the plate and incubated at 35˚ ± 2˚ C in ambient air and read after 8 hours of incubation with two independent reads performed.
Accelerate Pheno™ System: Using the same subculture plate utilized to make an inoculum for BMD, isolates were subcultured into human blood at a concentration of 10 to 100 CFU/mL and then inoculated into a BD Bactec™ Plus aerobic blood culture bottle and incubated on a BD Bactec™ FX blood culture system (BD, Sparks, MD). Species identification and AST were performed using the Accelerate PhenoTest™ BC kit run on the Accelerate Pheno™ system according to the manufacturer's instructions for use.
Reference Method: Broth microdilution was performed as the reference method according to CLSI using plates manufactured at Accelerate Diagnostics, Inc.
Data Analysis: CLSI M100S 28th Edition breakpoints were utilized for data analysis.
Categorical agreement (CA): isolates for which the categorical interpretation of the test method is the same as the reference method
Very major errors (VME): isolates for which the test method (dDD8 or AXDX) was susceptible and the reference method (BMD) was resistant
Major errors (ME): isolates for which the test method was resistant and the reference method was susceptible
Minor errors (mE): isolates for which either method reported a result as intermediate and the other method reported the result as susceptible or resistant

RESULTS

Table 1. Performance of test methods (AXDX, dDD8) compared to reference broth microdilution method.

				AXDX	dDD8	AXDX	dDD8	AXDX	dDD8	AXDX		dDD8	
	#S	#I	#R	CA (%)	CA (%)	VME(#)	VME(#)	ME(#)	ME(#)	mE(#)	mE(%)	mE(#)	mE(%)
Amikacin	39	0	3	100	97.6	0	0	0	0	0	0	1	2.4
Ampicillin	7	1	34	95.2	97.6	0	0	0	0	2	4.8	1	2.4
Ampicillin/sulbactam	17	4	21	83.3	78.6	0	3	2	2	5	11.9	4	9.5
Aztreonam	26	1	15	95.2	92.9	1	1	0	0	1	2.4	2	4.8
Cefazolin	13	4	25	88.1	69.0	0	1	0	1	5	11.9	11	26.2
Cefepime	24	3	15	90.5	95.2	0	0	0	0	4	9.5	2	4.8
Ceftazidime	26	1	15	92.9	95.2	1	1	0	0	2	4.8	1	2.4
Ceftriaxone	23	0	19	95.2	97.6	2	1	0	0	0	0	0	0
Ciprofloxacin	25	1	16	100	92.9	0	0	0	0	0	0	3	7.1
Ertapenem	36	0	6	100	100	0	0	0	0	0	0	0	0
Gentamicin	28	0	14	100	95.2	0	0	0	1	0	0	1	2.4
Meropenem	37	0	5	100	97.6	0	0	0	0	0	0	1	2.4
Piperacillin/tazobactam	36	1	5	95.2	88.1	0	0	1	0	1	2.4	5	11.9
Sulfamethox/trimeth	23	0	19	95.2	90.5	2	3	0	0	0	0	1	2.4
Tobramycin	28	4	10	90.5	95.2	0	0	1	0	3	7.1	2	4.8
Total						6	10	4	3	23	3.7	35	5.6

- Aztreonam, ceftazidime and tobramycin had the highest rates of inter-operator variability occurring in ≥10 isolates.
- Overall, inter-operator variability was observed 16.8% of the time for direct disk diffusion.

CONCLUSIONS

- The Accelerate Pheno™ system yielded CA results above 90% for 13 out of 15 antimicrobials evaluated, with all antimicrobials performing above 80%. 12 out of 15 antimicrobials tested via direct disk diffusion had CA above 90%.
- Ampicillin/sulbactam and cefazolin posed to be the most challenging across both methods, with CA of 78.6% and 69.0% for direct disk diffusion and 83.3% and 88.1% for the Accelerate Pheno™ system.
- The Accelerate Pheno™ system provides a comprehensive solution to clinical laboratories as an automated platform with an integrated ID method and a more extensive antimicrobial menu.
- Direct disk diffusion yielded acceptable performance but necessitates the use of manual interpretation and reading in most clinical laboratories, subjecting the results to more variability and potentially error. Additionally, due to the manual nature and specific incubation time requirements of the method, it is likely that it would necessitate batching for most clinical laboratories.
- Currently, direct disk diffusion is not a recognized method of susceptibility testing by CLSI. EUCAST recently published guidance, however for gram negative organisms it is limited to *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* with breakpoint guidance limited to the following antimicrobials piperacillin/tazobactam, cefotaxime, ceftazidime, meropenem, imipenem, ciprofloxacin, amikacin, gentamicin, and tobramycin.

REFERENCES

1. Zilberberg MD, et al. Crit Care. 2014 Nov 21; 18(6):596. 2. Chandrasekaran S, et al. JCM 2018 Feb 22; 56(3). 3. CLSI 2018 Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI Supplement M100S.