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

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MODIFIED ABSTRACT

Background: Antimicrobial susceptibility testing is an integral component of selecting effective antimicrobial therapy. Conventional AST can take 48-72 hours before results are available. Fast methods to shorten the time to result can potentially lead to improved patient outcomes.
Methods: AST was performed on a total of 53 isolates (20 *Pseudomonas aeruginosa* and 33 *Enterobacteriaceae* isolates with the following methods: broth microdilution (BMD), rapid disk diffusion with reading performed 8 hours (rDD8) after incubation, and the Accelerate Pheno™ system (AXDX). For rDD8 and AXDX, positive blood culture broth was utilized as inoculum. Categorical agreement (CA) was evaluated using the recommended 2018 CLSI 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 for AXDX, dDD8, and were 93.4%, 90.9%, respectively. AXDX had 6 VMEs and 4 MEs, dDD8 had 10 VMEs and 4 MEs, when compared with BMD. mE rates for AXDX and dDD8 were 5.1% and 7.0%, respectively.
Conclusions: All methods evaluated had CA above 90%. AXDX provided accurate susceptibility utilizing a fully automated method with streamlined workflow compared with manual methods such as dDD8. More resistant isolates are needed to elucidate the VME rates amongst the different methods evaluated in this data set.

BACKGROUND

Importance of early appropriate antimicrobial therapy for patients with suspected bloodstream infections is well established.[1]
Until recently, FDA cleared methods for antimicrobial 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 rapid 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 susceptibilities in approximately 7 hours directly from a positive blood culture. The rapid disk diffusion method also utilizes a positive blood culture in lieu of an inoculum generated from pure culture.[2]
The objective of this study was to evaluate the performance of two fast AST methods, rapid disk diffusion and the Accelerate Pheno™ system compared to reference broth microdilution (BMD).

METHODS

A total of 53 clinical isolates were evaluated (20 *Pseudomonas aeruginosa*, 33 *Enterobacteriaceae).*
Antimicrobial agents tested include 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), and tobramycin (10 µg).
Rapid 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. Rapid disk diffusion plates were 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 BACTEC™ Plus aerobic blood culture bottle and incubated on a 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 (RUO software).
Reference Method: Broth microdilution was performed as the reference method according to CLSI guidance. BMD plates were manufactured at Accelerate Diagnostics, Inc.
Data Analysis:
CLSI M100S 28th Edition breakpoints were utilized for data analysis to determine categorical and agreement and errors as follows:[3]
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 (rDD8 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. *Pseudomonas aeruginosa* performance of test methods (AXDX, rDD8) compared to reference broth microdilution method.

				CA (%)		VME (#)		ME (#)		mE (#)	
	#S	#I	#R	AXDX	rDD8	AXDX	rDD8	AXDX	rDD8	AXDX	rDD8
Amikacin	20	0	0	100	100	0	0	0	0	0	0
Aztreonam	16	2	2	84.2	33.3	0	0	0	0	3	12
Cefepime	18	1	1	94.4	100	0	0	0	0	1	0
Ceftazidime	18	0	2	84.2	94.4	1	0	0	0	2	1
Ciprofloxacin	20	0	0	94.8	100	0	0	0	0	1	0
Gentamicin	20	0	0	89.5	100	0	0	0	0	2	0
Meropenem	19	0	1	89.5	100	0	0	1	0	1	0
Piperacillin/tazobactam	18	1	1	73.7	83.3	0	0	0	0	5	3
Tobramycin	20	0	0	100	100	0	0	0	0	0	0
Total						1	0	1	0	15	16

AXDX evaluable isolates (n=19, 1 technical failure, except cefepime n=18 due to 1 additional failure); rDD8 evaluable isolates (n=18, 2 isolates too light to read)

Table 2 *Enterobacteriaceae* performance of test methods (AXDX, rDD8) compared to reference broth microdilution method.

				CA (%)		VME (#)		ME (#)		mE (#)	
	#S	#I	#R	AXDX	rDD8	AXDX	rDD8	AXDX	rDD8	AXDX	rDD8
Amikacin	33	0	0	100	100	0	0	0	0	0	0
Ampicillin	7	1	25	93.8	97.0	0	0	0	0	2	1
Ampicillin/sulbactam	17	3	13	78.1	75.8	0	3	2	2	5	3
Aztreonam	25	1	7	93.8	93.9	1	1	0	0	1	1
Cefazolin	13	4	16	84.4	60.6	0	1	0	1	5	11
Cefepime	24	1	8	93.8	93.9	0	0	0	0	2	2
Ceftazidime	25	0	8	93.8	93.9	1	1	0	0	1	1
Ceftriaxone	23	0	10	93.8	97.0	2	1	0	0	0	0
Ciprofloxacin	22	1	10	100	90.9	0	0	0	0	0	3
Ertapenem	33	0	0	100	100	0	0	0	0	0	0
Gentamicin	26	0	7	100	93.9	0	0	0	1	0	1
Meropenem	33	0	0	100	100	0	0	0	0	0	0
Piperacillin/tazobactam	33	0	0	96.9	84.8	0	0	1	0	0	5
Sulfamethoxazole/trimethoprim	21	0	12	96.9	87.9	1	3	0	0	0	1
Tobramycin	26	2	5	93.8	97.0	0	0	0	0	2	1
Total						5	10	3	4	18	30

AXDX evaluable isolates (n=32, 1 technical failure); rDD8 evaluable isolates (n=33)

CONCLUSIONS

- Overall CA was 93.4% and 90.9% for AXDX and rDD8, respectively.
- For *Pseudomonas aeruginosa* isolates tested with rapid disk diffusion, aztreonam had a very low CA rate (33%) due to many minor errors. Despite an 8-hour incubation period for the isolates tested with rapid disk diffusion, certain *Pseudomonas aeruginosa* isolates exhibited insufficient growth and therefore rendered no result.
- For *Enterobacteriaceae,* ampicillin/sulbactam and cefazolin were the lowest performing antimicrobials across both methods, with CA of 75.8% and 60.6% for rapid disk diffusion and 78.1% and 84.4% for the Accelerate Pheno™ system, respectively.
- Rapid disk diffusion had 10 VME and AXDX had 6 VME, however, it should be noted that the current data set does not have a sufficient number of resistant isolates to appropriately assess VME.
- As an automated platform with an integrated ID method and a simple integration into the clinical microbiology workflow, the Accelerate Pheno™ system provides an FDA cleared, comprehensive solution to clinical laboratories. Currently, rapid disk diffusion is not a CLSI recognized or FDA cleared method of susceptibility testing. Additionally, while rapid disk diffusion provides a categorical interpretation it does not provide a MIC result.

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.