Retrospective Evaluation of Accelerate Pheno™ System (AXDX, Version 1.1) for Identification and Antimicrobial Susceptibility Testing of Gram-Negative Bacilli including Carbapenemase-Producing Organisms (CPO) from Seeded Blood Culture Bottles Tested using AXDX PhenoTest™ BC Kits

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UPDATED ABSTRACT

Background: AXDX reports ID/AST in <7h from positive blood cultures. Prospective pre-FDA clinical trials were conducted in areas where CPO bacteremia was rare. This study challenged AXDX to detect CPO from SBCB.

Methods: 53 GNB including 35 CPO (10 KPC, 8 OXA48-type, 4 NDM, 3 GES5, 3 VIM, 1 VIM/GES5, 1 IMP7, 1 SME) were tested in AXDX post SBCB incubation in BacT/Alert® (bioMérieux). Seeding suspensions were parallel-tested by CLSI M100-S27 broth microdilution (BMD) for cefazolin (CFZ), ceftriaxone (CRO), ceftazidime (CAZ), piperacillin/tazobactam (TZP), ertapenem (ETP), meropenem (MEM), ciprofloxacin (CIP), gentamicin (GM), tobramycin (TOB), amikacin (AN), and colistin (CST). With GNB-genera combined, AXDX-AST were assessed vs BMD using Cumitech 31A for ≥90% agreements [essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%].

Results: AXDX produced evaluable results (ID-correct/AST-reported) for the 44 (83%) GNB (19 *Klebsiella* species, 13 *Escherichia coli*, 5 *Pseudomonas aeruginosa*, 4 *Enterobacter cloacae*, 2 *Proteus mirabilis*, 1 *Serratia marcescens*) tabulated below. Limits were exceeded for underlined values, but 95% confidence intervals (CI) overlapped acceptable limits except in values marked with asterisks [95%CI: EA (CRO:58.8-85.6; ETP:31.6-61.4; MEM:62.8-87.3; GEN:48.8-76.3); VME (MEM:11.7-45.2); ME/mE (CAZ:7.8-30.3)].

Evaluable (n=44)	CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AMK	CST
AXDX-AST (no.)	32	39	43	44	39	44	44	44	44	41	41
BMD-S (no.)	1	7	9	9	10	19	14	23	17	27	33
BMD-I (no.)	1	1	1	7	5	1	2	1	2	4	0
BMD-R (no.)	30	31	33	28	24	24	28	20	25	10	8
%EA	93.8	74.4*	81.4	81.8	46.2*	77.3*	93.2	63.6*	93.2	87.8	97.6
% CA	96.9	84.6	83.7	86.4	87.2	81.8	95.5	88.6	88.6	85.4	97.6
%VME	0	6.5	0	0	4.2	25*	0	0	0	10	12.5
%ME/mE	0/3.1	0/10.3	0/16.3*	0/13.6	0/10.3	0/4.6	0/4.6	13/4.6	5.9/9.1	0/12.2	0/0

Of 31 CPO, by BMD/AXDX respectively, 2 (6.5%; *P. mirabilis* NDM, *E. coli* KPC)/4 (12.9%; same 2 plus *E. cloacae* KPC, *S. marcescens* SME) were ETP+MEM=S, 23 (74.2%)/17 (54.8%) were ETP+MEM=I/R and 6 (19.4%; 5 OXA48; 1 KPC)/12 (38.7%; same plus 4 KPC, 1 NDM, 1 SME) were ETP=I/R but MEM=S. If ETP-I/R alone predicted CPO, BMD/AXDX detected 93.5% (95%CI: 78.3-99.2)/87.1% (95%CI: 70.5-95.5) CPO, respectively (P=0.6713, NS). But as 3/5 (60%) ETP-R non-CPO were also MEM=S by BMD+AXDX, this rule would incur MEM ME.

Conclusions: Expert rules based on ETP enabled detection of 87.1% (70.5-95.5%) CPO by AXDX in <7h. But while this rule mitigates MEM VME, it risks introducing MEM ME. Further optimization of AXDX algorithms to distinguish challenging CPO growth patterns associated with low carbapenem MIC from non-CPO is underway. More CPO/non-CPO should be tested to tighten 95%CI obtained in this promising study.

INTRODUCTION

To reduce mortality, morbidity and length of stay in acutely ill patients, especially in bacteremia complicated by Gram-negative bacilli (GNB) septic shock, it is essential to initiate therapy without delay. In such patients, treatment is started before the etiological agent is known, typically using broad-spectrum empiric regimens that may or may not be appropriate. Timeliness of subsequent escalation/de-escalation to achieve organism-specific tailored therapy, depends on laboratory turn-around-time (TAT) of organism identifications (ID) and antimicrobial susceptibility test (AST) results. When multidrug-resistant (MDR) GNB such as carbapenemase-producing organisms (CPO) are isolated, minimum inhibitory concentrations (MIC) may be needed for full optimization of therapy.

In recent years, most clinical microbiology laboratories have significantly reduced ID-TAT by implementing MALDI-TOF mass spectrometry (MS). From blood cultures (BC), ID-TAT has been further optimized by performing MS on first visible growth from short-incubation BC isolates, reducing ID-TAT to ~4-6h post Gram stain report. But, as current AST methods have been in place for >20 years, AST are only reported 24-72h after ID. Thus much impetus has been invested in finding alternative methods to similarly improve AST-TAT.

To meet this need, Accelerate Diagnostics (AXDX, Tucson, Arizona, US) designed an innovative low-complexity system to deliver ID/AST with MIC directly from BCs in <7h. Its simple set-up requires only for blood from a newly-positive BC to be transferred to a vial that is placed into a AXDX BC PhenoTest™ kit cartridge, which together with a test cassette, are inserted into the AXDX Pheno™ system for testing. After processing to separate organisms from interfering blood products, Acridine Orange detects all bacteria/yeast present to enable mono- vs. poly-microbial calls, after which fluorescent *in situ* hybridization probes proceed to ID a select menu of organisms common in septicemia. When a bacterial ID is achieved (~1h20m), the remaining cells are distributed to cassette wells where they are immobilized onto fine agar layers containing specific antibiotics for AST. Using precise coordinates, each bacterial cell is monitored for growth/time using automated microscopic imaging. After ~5h, graphic comparison of cellular behavior of the test organism against historic behavior amassed in the AXDX database from similar organisms exposed to each antibiotic allows AXDX to predict specific a MIC for each bacteria/drug combination. MIC are translated into susceptibility/resistance (S/R) categories using CLSI M100-S27. Thus, AXDX provides ID with MIC for a comprehensive range of antimicrobials in <7h from time of BC+/Gram stain - significantly quicker TAT compared to even the most optimized current ID/AST protocols.

This retrospective study compared ID/AST by AXDX to gold standard MS and broth microdilution (BMD). To compliment prospective AXDX testing, resistance genotypes (i.e. CPO) that are emerging but not yet common in Toronto, Canada bacteremia patients were selected. BacT/Alert® FA Plus bottles were seeded and incubated by BacT/Alert® systems (bioMérieux) to simulate patient BC, and tested by AXDX when positive.

METHODS

- Table 1 (below) lists major characteristics of 53 retrospective GNB selected for evaluation. These comprised 17 *Escherichia coli*, 16 *Klebsiella pneumoniae*, 5 *Pseudomonas aeruginosa*, 4 *Enterobacter cloacae*, 4 *K. oxytoca*, 2 *Proteus mirabilis*, 1 ea. *Citrobacter freundii*, *E. asburiae*, *K. ozaenae*, *Serratia marcescens*, *P. putida*. Genotypes of interest included 35 CPO [14 *blaKPC* , 8 *blaOXA*$_{48}$-type, 4 *blaNDM* , 3 *blaGES*$_5$, 3 *blaVIM* , 1 *blaVIM/GES*$_5$, 1 *blaIMP*$_7$, 1 *blaSME*] while 4 *E. coli* carried the *mcr1* colistin resistance gene (resistance determinants for other antibiotic classes not listed).
- To prevent plasmid loss, selective pressure was maintained on isolate recovery from -80°C, during 2nd subcultures and on purity plate after ID/AST testing by placing ertapenem discs on culture agars. Ertapenem zones were recorded at all stages to demonstrate continued presence/absence of carbapenemase genes.
- Before study testing, MALDI-TOF MS (VITEK® MS Plus, bioMérieux) was performed to confirm organism ID.
- From each confirmed isolate, 75 CFU was seeded to a BacT/Alert® FA Plus bottle containing 10mL human blood and incubated immediately in the BacT/Alert® 3D system (bioMérieux) until flagged as positive.
- Within 8h of the seeded BC bottle being flagged positive by the BacT/Alert®, an aliquot of blood-broth was transferred directly from the BC bottle to an Accelerate PhenoTest™ BC kit sample vial for testing. Each sample vial is clipped into a reagent cartridge and inserted together with a cassette into the Accelerate Pheno™ system for automated ID/AST determinations.
- All 0.5 MacFarland bacterial suspensions used to seed BC bottles were also tested by AXDX broth microdilution (BMD) as per CLSI. BMD included cefazolin (CFZ), ceftriaxone (CRO), ceftazidime (CAZ), piperacillin/tazobactam (TZP), ertapenem (ETP), meropenem (MEM), ciprofloxacin (CIP), gentamicin (GM), tobramycin (TOB), amikacin (AN), and colistin (CST) enabling comparison for all AXDX-AST results.
- AXDX-BMD MIC were recorded independently by 3 readers; consensus results were interpreted as per CLSI M100-S27. If consensus was not obtained, the BMD panel was imaged and re-checked by an expert.
- With GNB-genera combined, available AXDX-AST were compared to BMD as per Cumitech 31A guidelines for ≥90% agreements [essential (EA); categorical (CA)] and errors [very major (VME) <3%; combined major/minor (ME/mE) <7%]. 95% confidence intervals (CI) were calculated online using www.graphpad/quickcalcs.

Table 1. Characteristics of 53 Gram-negative bacilli including 35 carbapenemase-producing organisms (CPO) used to simulate blood cultures for evaluation of ID/AST accuracy of the Accelerate Pheno™ system (AXDX version 1.1) using AXDX PhenoTest™ BC kits

CPO Amber class (No.)	Genotypes (No.)	Genus/Species identification by MALDI-TOF MS (No.)	No. of isolates correctly identified by AXDX (other ID listed)	Total with AXDX ID and AST (n=44)
A (18)	*blaKPC* (14)	*Citrobacter freundii* (1)	1 (also *Klebsiella* spp. positive)	0
		Enterobacter cloacae (1)	1 (also *S. aureus* positive)	1
		Escherichia coli (3)	1 (2 with no ID)	1
		Klebsiella pneumonia (9)	8 (1 with no ID)	8
	blaGES5 (3)	*Klebsiella oxytoca* (3)	3 (1 *S. lugdunensis* indeterminate)	3
	blaSME1/4 (1)	*Serratia marcescens* (1)	1	1
A+ B (1)	*blaGES5-blaVIM* (1)	*Pseudomonas aeruginosa* (1)	1	1
B (8)	*blaIMP7* (1)	*Pseudomonas aeruginosa* (1)	1	1
	blaNDM (4)	*Enterobacter cloacae* (1)	1	1
		Escherichia coli (2)	2	2
		Proteus mirabilis (1)	1	1
	blaVIM (3)	*Enterobacter cloacae* (1)	1	1
		Pseudomonas aeruginosa (2)	2 (1 CNST indeterminate)	2
D (8)	*blaOXA48* (4; 1 *mcr1*)	*Escherichia coli* (4; 1 *mcr1*)	4	4
	blaOXA181 (2)	*Escherichia coli* (1)	1	1
		Klebsiella pneumoniae (1)	1	1
	blaOXA232 (2)	*Klebsiella pneumoniae* (2)	2	2
Non-CPO (18)	MDR: *CTX-M15, CMY2 ampC +/- ompC/F* (3)	*Escherichia coli* (3)	3	3
	MDR: *ompC/F* (1)	*Enterobacter asburiae* (1)	0 (*Enterobacter* indeterminate)	0
	XDR *ompK35/36* (1)	*Klebsiella pneumoniae* (1)	1	1
	XDR CRE (1)	*Pseudomonas aeruginosa* (1)	1	1
	Colistin-R (7; 3 *mcr1*)	*Enterobacter cloacae* (1)	1	1
		Escherichia coli (4; 3 *mcr1*)	2 (2 *mcr1* with no ID)	2
		Klebsiella pneumoniae (2)	2 (1 ea. *Enterobacter*/CNST indeterminate)	2
	Non-MDR (3)	*Klebsiella pneumoniae* (1)	1	1
		Klebsiella oxytoca (1)	1 (*S. lugdunensis* indeterminate)	1
		Proteus mirabilis (1)	1	1
	ID off-panel (2)	*Klebsiella ozaenae* (1)	0	0
		Pseudomonas putida (1)	0	0

RESULTS

Of 53 GNB tested, 51 were on the AXDX ID menu. Of these 51, 44 (86.3%), including 31/35 (88.6%) CPO, were evaluable as AXDX produced a correct ID and corresponding AST (Table 1). As isolate numbers per species were too low to evaluate each individually, results were combined across genera for comparison of AXDX AST to BMD AST results (Table 2).

AXDX performance compared to BMD AST per CLSI

- **AXDX essential agreement (EA) results**
  - Antimicrobials meeting acceptible >90% EA imits are highlighted green
  - Antimicrobials NOT meeting acceptible EA limits BUT within 95% CI acceptible limit ranges are highlighted yellow
  - Antimicrobials outside 95% CI acceptible limit ranges are highlighted red i.e. those outside 95%CI ranges for EA (CRO: 58.8-85.6%; ETP: 31.6-61.4%; MEM: 62.8-87.3%; GEN: 48.8-76.3%)
- **AXDX categorical agreement (CA) results**
  - Antimicrobials meeting acceptible >90% limits for CA are highlighted in green
  - Antimicrobials NOT meeting acceptible CA limits BUT within 95% CI acceptible limit ranges are highlighted yellow
- **AXDX very major error (VME) results**
  - Antimicrobials meeting acceptible <3% VME limits are highlighted green
  - Antimicrobials NOT meeting the acceptible limit BUT within 95% CI limit ranges are highlighted yellow
  - Meropenem, highlighted red, was the only AXDX agent outside 95% CI acceptible VME limit ranges (11.7-45.2%)
- **AXDX combined major and minor error (ME/mE) results**
  - While ME and mE data are displayed separately in Table 2, the acceptible limit is <7% for ME/mEcombined
  - Antimicrobials meeting acceptible ME/mE limits are highlighted green
  - Antimicrobials NOT meeting acceptible limits BUT combined values fell within 95% CI are highlighted yellow
  - Ceftazidime, highlighted red, was the only AXDX agent outside 95% CI acceptible ME/mE limit ranges (7.8-30.3%)

Table 2. Summary of performance criteria for 44 retrospective Gram-negative bacilli with evaluable AXDX ID/AST results tested directly from positive blood culture bottles where AXDX AST results are compared to MALDI-TOF MS identifications (ID) and CLSI broth microdilution (BMD; S, I and R distributions shown) reference AST (results show Gram-negative genera combined)

Antibiotic	CFZ	CRO	CAZ	TZP	ETP	MEM	CIP	GEN	TOB	AMK	CST
No. AXDX-AST	32	39	43	44	39	44	44	44	44	41	41
No. S by BMD	1	7	9	9	10	19	14	23	17	27	33
No. I by BMD	1	1	1	7	5	1	2	1	2	4	0
No. R by BMD	30	31	33	28	24	24	28	20	25	10	8
AXDX EA	30 (93.8)	29 (74.4)	35 (81.4)	36 (81.8)	18 (46.2)	34 (77.3)	41 (93.2)	28 (63.6)	41 (93.2)	36 (87.8)	40 (97.6)
No. (%) 95% CI	78.8-99.3	58.8-85.6	67.1-90.5	67.8-90.6	31.6-61.4	62.8-87.3	81.1-98.3	48.8-76.3	81.1-98.3	74-95.1	86.3->99.9
AXDX CA	31 (96.9)	33 (84.6)	36 (83.7)	38 (86.4)	34 (87.2)	36 (81.8)	42 (95.5)	39 (88.6)	39 (88.6)	35 (85.4)	40 (97.6)
No. (%) 95% CI	82.9->99.9	69.9-93.1	69.7-92.2	72.9-94	72.8-94.9	67.8-90.8	84.1-99.6	75.6-95.5	75.6-95.5	71.2-93.5	86.3->99.9
AXDX VME	0	2 (6.5)	0	0	1 (4.2)	6 (25)	0	0	0	1 (10)	1 (12.5)
No. (%) 95% CI	0-13.5	0.8-21.8	0-12.4	0-14.3	<0.01-21.9	11.7-45.2	0-14.3	0-19	0-15.8	<0.01-42.6	0.1-49.2
AXDX ME	0	0	0	0	0	0	0	3 (13)	1 (5.9)	0	0
No. (%) 95% CI	0-83.3	0-40.4	0-34.5	0-34.5	0-32.1	0-19.8	0-25.2	3.7-33	<0.01-28.9	0-14.8	0-12.4
AXDX mE	1 (3.1)	4 (10.3)	7 (16.3)	6 (13.6)	4 (10.3)	2 (4.6)	2 (4.6)	2 (4.6)	4 (9.1)	5 (12.2)	0
No. (%) 95% CI	<0.01-17.1	3.5-24.2	7.8-30.3	6-27.1	3.5-24.2	04-16	0.4-16	0.4-16	3-21.7	4.9-26	0-10.2

AXDX and BMD performance for CPO assuming CPO should be considered carbapenem-resistant

Of 31 CPO with AXDX ID/AST, BMD and AXDX ETP and MEM MIC results compared as follows:

- CPO with ETP/MEM=R/R or R/I: 23 (74.2%) by BMD vs. 17 (54.8%) by AXDX
- CPO with ETP/MEM=S/S: 2 (6.5%) by BMD (*P. mirabilis*-NDM, *E. coli*-KPC) vs. 4 (12.9%) by AXDX (same 2, + *E. cloacae*-KPC, *S. marcescens* SME)
- CPO with ETP=I/R but MEM=S: 6 (19.4%) by BMD (5 OXA48; 1 KPC) vs. 12 (38.7%) by AXDX (same 6, + 4 KPC, 1 NDM, 1 SME)

When ETP=I/R alone was used to predict CPO, detection by BMD was 93.5% (95%CI: 78.3-99.2) vs. 87.1% (95%CI: 70.5-95.5) by AXDX (P=0.6713, NS). But as 3/5 (60%) ETP=R non-CPO were also MEM=S by BMD and AXDX, this rule would incur MEM ME.

CONCLUSIONS

- **To complement the prospective AXDX evaluation, where few resistant GNB were tested, this retrospective study included 70% CPO to challenge AXDX abilities for detecting emerging genotypes.**
- **The AXDX PhenoTest™ BC kit and Pheno™ system were extremely simple to use and provided rapid ID/AST with MIC in 86.3% seeded BC tested directly from the BacT/Alert® bottles, with ID/AST reporting at 1.3h/6.5h , respectively.**
- **Acceptable performance was obtained for most antimicrobials with the exception of 25% VME noted for MEM and 16.3% mE noted with CAZ. EA but not CA was also outside limits for ETP, MEM, CRO, and GEN. New software versions with expert rules have been developed in an attempt to mitigate errors. For example, AXDX expert rules based on ETP/MEM R/S discrepancies have been included in AXDX version 1.2.1 that mitigate most MEM VME by reporting ETP-R but suppressing potentially discrepant MEM results and requiring that MEM AST be determined by an alternative method in order to prevent introducing MEM ME.**
- **More CPO/non-CPO should be tested to verify expert rules and tighten 95%CI obtained in this promising study.**

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