A new rapid antimicrobial susceptibility testing assay evaluated with positive blood cultures for antimicrobial-resistant bacterial pathogens

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BACKGROUND

Early determination of antimicrobial susceptibility testing (AST) profiles for bacterial organisms that cause bloodstream infection (BSI) is crucial to reduce the empirical use of broad-spectrum antibiotics and, meanwhile, improve timing and efficacy of antimicrobial therapy. However, conventional AST methods, such as broth microdilution (BMD) assays, may take 24–48 h to provide AST results because they are conceived to work on BSI isolates, i.e., organisms derived from subculture of a positive blood culture (PBC) sample.

OBJECTIVE

We evaluated a new rapid AST assay, the VITEK® REVEAL™ (bioMérieux) system, which detects the growth of Gram-negative (GN) bacteria via their emission of volatile organic compounds directly from PBC samples.

METHODS

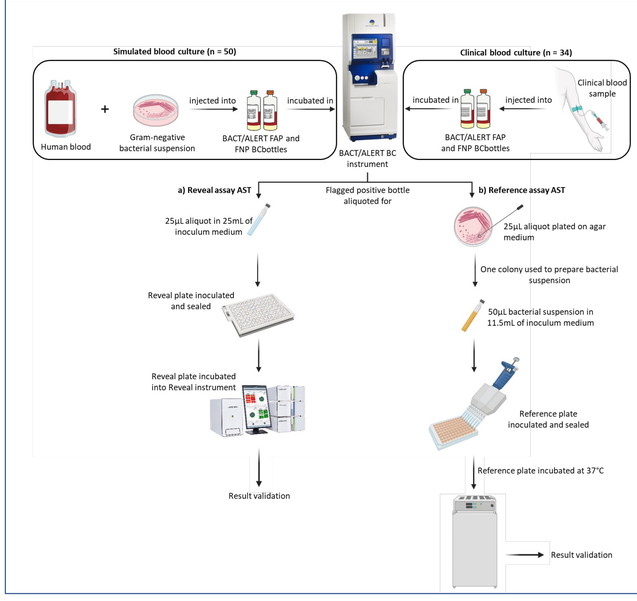


Figure 1. Antimicrobial susceptibility testing workflow.

We studied two (simulated or clinical) sets of PBC samples. The first set consisted of PBC samples resulting from the inoculation of blood culture (BC) bottles with GN (33 *Enterobacterales*, 11 *Pseudomonas aeruginosa*, and 6 *Acinetobacter baumannii* complex) organisms, selected to represent highly diversified resistance profiles (one per bottle). The second set consisted of PBC samples prospectively obtained from hospitalized patients, that grew GN (27 *Enterobacterales*, 6 *P. aeruginosa*, and 1 *A. baumannii* complex) organisms with antimicrobial-susceptible or -resistant profiles. Aliquots from each PBC bottle were used directly to perform the VITEK® REVEAL™ (GN01) AST assay and were plated on solid media, and overnight-grown isolates were used for broth microdilution-based AST reference assay (Figure 1). Minimum inhibitory concentration (MIC) values were interpreted using the EUCAST 2024 clinical breakpoints of antibiotics, and discrepancies were calculated according to ISO-criteria 20776/2:2007.

RESULTS

We tested 683 and 564 combinations of bacterial organisms and antibiotics for simulated or clinical PBC samples, respectively. As shown in Tables 1 and 2 and, only for new β-lactam-β-lactamase inhibitor combinations, in Figure 2, rates of essential agreement (EA) and categorical agreement (CA) of the VITEK® REVEAL™ with the reference assay were, respectively, 95.0% and 96.2% for GN organisms from simulated PBC samples (n = 50) and 98.2% and 98.9% for GN organisms from clinical PBC samples (n = 34). Very major discrepancies (VMDs) were observed for simulated (10/451; 2.2%) and clinical (1/116; 0.9%) PBC samples. Major discrepancies were only observed for simulated (3/308; 1.0%) PBC samples. Two discrepancy results, which regarded antibiotics with no defined susceptible-increased exposure category, were in EA. Repeat testing led to resolve 1 VMD, which regarded a ceftolozane-tazobactam/*Klebsiella pneumoniae* combination in 1 simulated PBC sample.

Table 1. Performance of the VITEK® REVEAL™ AST assay for 50 GN organisms from simulated PBC samples

GN organisms tested against the indicated antibiotics	% of GN organisms categorised using BMD as: R (n)	I (n)	S (n)	% EA (n results/n tested)	% CA (n results/n tested)	% MD (n results/n tested)	% VMD (n results/n tested)	% mD (n results/n tested)
***Enterobacterales* (n = 33)**								
• Amikacin	11	-	22	100 (33/33)	100 (33/33)	0 (0/22)	0 (0/11)	-
• Amoxicillin/Clavulanic acid	28	-	3	96.77 (30/31)	100 (31/31)	0 (0/3)	0 (0/28)	-
• Aztreonam	21	2	9	93.75 (30/32)	93.75 (30/32)	0 (0/9)	0 (0/21)	6.25 (2/32)
• Cefepime	18	6	7	96.77 (30/31)	96.77 (30/31)	0 (0/7)	0 (0/18)	3.23 (1/31)
• Ceftazidime	28	2	3	84.84 (28/33)	87.88 (29/33)	0 (0/3)	0 (0/28)	12.12 (4/33)
• Ceftazidime-avibactam	8	-	25	100 (33/33)	100 (33/33)	0 (0/25)	0 (0/8)	-
• Cefotaxime	27	0	6	96.97 (32/33)	96.97 (32/33)	0 (0/6)	0 (0/27)	3.03 (1/33)
• Ceftolozane-tazobactam	21	-	11	87.50 (28/32)	93.75 (30/32)	0 (0/11)	9.52 (2/21)	-
• Ciprofloxacin	24	0	5	100 (29/29)	100 (29/29)	0 (0/5)	0 (0/24)	0 (0/29)
• Ertapenem	15	-	18	81.82 (27/33)	84.85 (28/33)	11.11 (2/18)	20 (3/15)	-
• Gentamicin	14	-	19	93.94 (31/33)	96.97 (32/33)	0 (0/19)	7.14 (1/14)	-
• Levofloxacin	22	1	10	100 (33/33)	100 (33/33)	0 (0/10)	0 (0/22)	0 (0/33)
• Imipenem	10	0	22	100 (32/32)	100 (32/32)	0 (0/22)	0 (0/10)	0 (0/32)
• Meropenem	9	1	23	84.85 (28/33)	87.88 (29/33)	0 (0/23)	0 (0/9)	12.12 (4/33)
• Meropenem-vaborbactam	6	-	27	100 (33/33)	100 (33/33)	0 (0/27)	0 (0/6)	-
• Piperacillin-tazobactam	23	-	9	84.38 (27/32)	93.75 (30/32)	0 (0/9)	8.70 (2/23)	-
• Tobramycin	21	0	12	100 (33/33)	100 (33/33)	0 (0/12)	0 (0/21)	0 (0/33)
• Trimethoprim-sulfamethoxazole	18	1	14	87.88 (29/33)	87.88 (29/33)	7.14 (1/14)	0 (0/18)	9.09 (3/33)
• Total antibiotics	324	13	245	93.81 (546/582)	95.53 (553/582)	1.22 (3/245)	2.47 (8/324)	4.66 (15/322)
***Pseudomonas aeruginosa* (n = 11)**								
• Amikacin	2	-	9	100 (11/11)	100 (11/11)	0 (0/9)	0 (0/2)	-
• Aztreonam	1	10	-	100 (11/11)	100 (11/11)	0 (0/10)	0 (0/1)	-
• Cefepime	8	3	-	100 (11/11)	100 (11/11)	0 (0/3)	0 (0/8)	-
• Ceftazidime	7	4	-	100 (11/11)	100 (11/11)	0 (0/4)	0 (0/7)	-
• Ceftazidime-avibactam	6	-	5	90.91 (10/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Ceftolozane-tazobactam	6	-	5	100 (11/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Ciprofloxacin	9	1	-	100 (10/10)	100 (10/10)	0 (0/1)	0 (0/9)	-
• Levofloxacin	9	2	-	100 (11/11)	100 (11/11)	0 (0/2)	0 (0/9)	-
• Imipenem	11	0	-	100 (11/11)	100 (11/11)	-	0 (0/11)	-
• Meropenem	8	3	0	100 (11/11)	100 (11/11)	0 (0/3)	0 (0/8)	0 (0/11)
• Meropenem-vaborbactam	9	-	2	90.91 (10/11)	100 (11/11)	0 (0/2)	0 (0/9)	-
• Piperacillin-tazobactam	6	5	-	90.91 (10/11)	81.82 (9/11)	0 (0/5)	33.33 (2/6)	-
• Tobramycin	6	-	5	100 (11/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Total antibiotics	88	28	26	97.89 (139/142)	98.59 (140/142)	0 (0/54)	2.27 (2/88)	0 (0/11)
***Acinetobacter baumannii complex* (n = 6)**								
• Amikacin	3	-	3	100 (6/6)	100 (6/6)	0 (0/3)	0 (0/3)	-
• Ciprofloxacin	6	0	-	100 (6/6)	100 (6/6)	-	0 (0/6)	-
• Gentamicin	3	-	3	100 (6/6)	100 (6/6)	0 (0/3)	0 (0/3)	-
• Levofloxacin	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Imipenem	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Meropenem	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Trimethoprim-sulfamethoxazole	5	0	1	100(6/6)	83.33 (5/6)	0 (0/1)	0 (0/5)	16.67 (1/6)
• Tobramycin	4	0	2	100 (6/6)	100 (6/6)	0 (0/2)	0 (0/4)	0 (0/6)
• Total antibiotics	39	0	9	100 (48/48)	97.92 (47/48)	0 (0/9)	0 (0/39)	3.33 (1/30)
All organisms (n = 50)	**451**	**41**	**280**	**94.95 (733/772)**	**96.24 (743/772)**	**0.97 (3/308)**	**2.22 (10/451)**	**4.41 (16/363)**

GN, gram negative; R, resistant; I, susceptible increased exposure; S, susceptible standard dosing regimen; EA, essential agreement; CA, categorical agreement; MD, major discrepancies; VMD, very major discrepancies; mD, minor discrepancies.

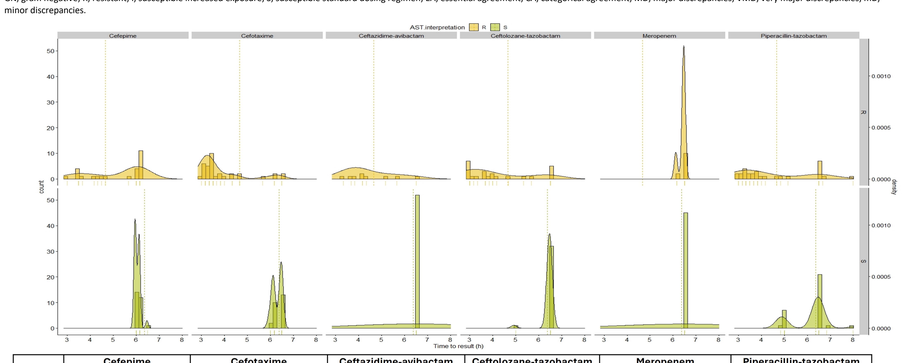
Table 2. Performance of the VITEK® REVEAL™ AST assay for 34 GN organisms from clinical PBC samples

GN organisms tested against the indicated antibiotics	% of GN organisms categorised using BMD as: R (n)	I (n)	S (n)	% EA (n results/n tested)	% CA (n results/n tested)	% MD (n results/n tested)	% VMD (n results/n tested)	% mD (n results/n tested)
***Enterobacterales* (n = 27)**								
• Amikacin	4	-	23	96.30 (26/27)	100 (27/27)	0 (0/23)	0 (0/4)	-
• Amoxicillin/Clavulanic acid	11	-	16	100 (27/27)	100 (27/27)	0 (0/16)	0 (0/11)	-
• Aztreonam	7	0	18	100 (25/25)	100 (25/25)	0 (0/18)	0 (0/7)	0 (0/25)
• Cefepime	7	0	20	92.59 (25/27)	100 (27/27)	0 (0/20)	0 (0/7)	0 (0/27)
• Ceftazidime	7	2	18	92.59 (25/27)	92.59 (25/27)	0 (0/18)	0 (0/7)	7.41 (2/27)
• Ceftazidime-avibactam	2	-	25	96.30 (26/27)	96.30 (26/27)	0 (0/25)	50.00 (1/2)	-
• Cefotaxime	7	0	20	100 (27/27)	100 (27/27)	0 (0/20)	0 (0/7)	0 (0/27)
• Ceftolozane-tazobactam	5	-	20	100 (25/25)	100 (25/25)	0 (0/20)	0 (0/5)	-
• Ciprofloxacin	8	0	17	100 (25/25)	96.00 (24/25)	0 (0/17)	0 (0/8)	4.00 (1/25)
• Ertapenem	4	-	23	100 (27/27)	100 (27/27)	0 (0/23)	0 (0/4)	-
• Gentamicin	6	-	21	100 (27/27)	100 (27/27)	0 (0/21)	0 (0/6)	-
• Levofloxacin	8	2	17	100 (27/27)	96.30 (26/27)	0 (0/17)	0 (0/8)	3.70 (1/27)
• Imipenem	4	0	21	96.00 (24/25)	96.00 (24/25)	0 (0/21)	0 (0/4)	4.00 (1/25)
• Meropenem	4	0	23	100 (27/27)	100 (27/27)	0 (0/23)	0 (0/4)	0 (0/27)
• Meropenem-vaborbactam	1	-	26	100 (27/27)	100 (27/27)	0 (0/26)	0 (0/1)	-
• Piperacillin-tazobactam	7	-	20	100 (27/27)	100 (27/27)	0 (0/20)	0 (0/7)	-
• Tobramycin	5	-	22	100 (27/27)	100 (27/27)	0 (0/22)	0 (0/5)	-
• Trimethoprim-sulfamethoxazole	9	0	18	100 (27/27)	100 (27/27)	0 (0/18)	0 (0/9)	0 (0/27)
• Total antibiotics	106	4	368	98.54 (471/478)	98.74 (472/478)	0 (0/368)	0.94 (1/106)	2.11 (5/237)
***Pseudomonas aeruginosa* (n = 6)**								
• Amikacin	0	-	6	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Aztreonam	0	6	-	83.33 (5/6)	100 (6/6)	0 (0/6)	-	-
• Cefepime	1	5	-	100 (6/6)	100 (6/6)	0 (0/5)	0 (0/1)	-
• Ceftazidime	0	6	-	83.33 (5/6)	100 (6/6)	0 (0/6)	-	-
• Ceftazidime-avibactam	0	-	6	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Ceftolozane-tazobactam	0	-	6	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Ciprofloxacin	1	5	-	100 (6/6)	100 (6/6)	0 (0/5)	0 (0/1)	-
• Levofloxacin	0	6	-	83.33 (5/6)	100 (6/6)	0 (0/6)	-	-
• Imipenem	0	6	-	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Meropenem	0	0	6	100 (6/6)	100 (6/6)	0 (0/6)	-	0 (0/6)
• Meropenem-vaborbactam	0	-	6	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Piperacillin-tazobactam	0	6	-	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Tobramycin	0	-	6	100 (6/6)	100 (6/6)	0 (0/6)	-	-
• Total antibiotics	2	40	36	96.15 (75/78)	100 (78/78)	0 (0/76)	0 (0/2)	0 (0/6)
***Acinetobacter baumannii complex* (n = 1)**								
• Amikacin	1	-	0	100 (1/1)	100 (1/1)	-	0 (0/1)	-
• Ciprofloxacin	1	0	-	100 (1/1)	100 (1/1)	-	0 (0/1)	-
• Gentamicin	1	-	0	100 (1/1)	100 (1/1)	-	0 (0/1)	-
• Levofloxacin	1	0	0	100 (1/1)	100 (1/1)	-	0 (0/1)	0 (0/1)
• Imipenem	1	0	0	100 (1/1)	100 (1/1)	-	0 (0/1)	0 (0/1)
• Meropenem	1	0	0	100 (1/1)	100 (1/1)	-	0 (0/1)	0 (0/1)
• Trimethoprim-sulfamethoxazole	1	0	0	100 (1/1)	100 (1/1)	-	0 (0/1)	0 (0/1)
• Tobramycin	1	0	0	100 (1/1)	100 (1/1)	-	0 (0/1)	0 (0/1)
• Total antibiotics	8	0	0	100 (8/8)	100 (8/8)	-	0 (0/8)	0 (0/5)
All organisms (n = 34)	**116**	**44**	**404**	**98.22 (554/564)**	**98.94 (558/564)**	**0 (0/444)**	**0.86 (1/116)**	**2.02 (5/248)**

GN, gram negative; R, resistant; I, susceptible increased exposure; S, susceptible standard dosing regimen; EA, essential agreement; CA, categorical agreement; MD, major discrepancies; VMD, very major discrepancies; mD, minor discrepancies.

A workflow analysis revealed a mean (±SD) time to result (calculated from the time a BC flagged positive) of 7.48 (±0.21) hours for the VITEK® REVEAL™ and 39.07 (±0.16) hours for the reference assay. Considering *Enterobacterales* organisms from both simulated and clinical PBC samples (n = 60), we found that mean time-to-result values, calculated by single antibiotics, were always shorter in PBC samples for resistant GN organisms than in PBC samples for susceptible GN organisms (see Figure 3 for the most clinically relevant antibiotics).

Figure 3. Times to VITEK® REVEAL™ AST assay results for *Enterobacterales* organisms from simulated or clinical PBC samples (n = 60). Values (expressed in hours) are stratified according to whether the organisms tested as susceptible or resistant to indicated antibiotics.



	Cefepime		Cefotaxime		Ceftazidime-avibactam		Ceftolozane-tazobactam		Meropenem		Piperacillin-tazobactam	
	R	S	R	S	R	S	R	S	R	S	R	S
No.	26	27	35	25	9	52	24	33	12	45	29	31
Mean (h)	05:13	06:05	03:56	06:20	04:32	06:30	04:17	06:27	06:26	06:30	04:38	06:10
SD(h)	01:10	00:05	01:01	00:11	01:02	00:00	01:21	00:15	00:07	00:00	01:30	00:46

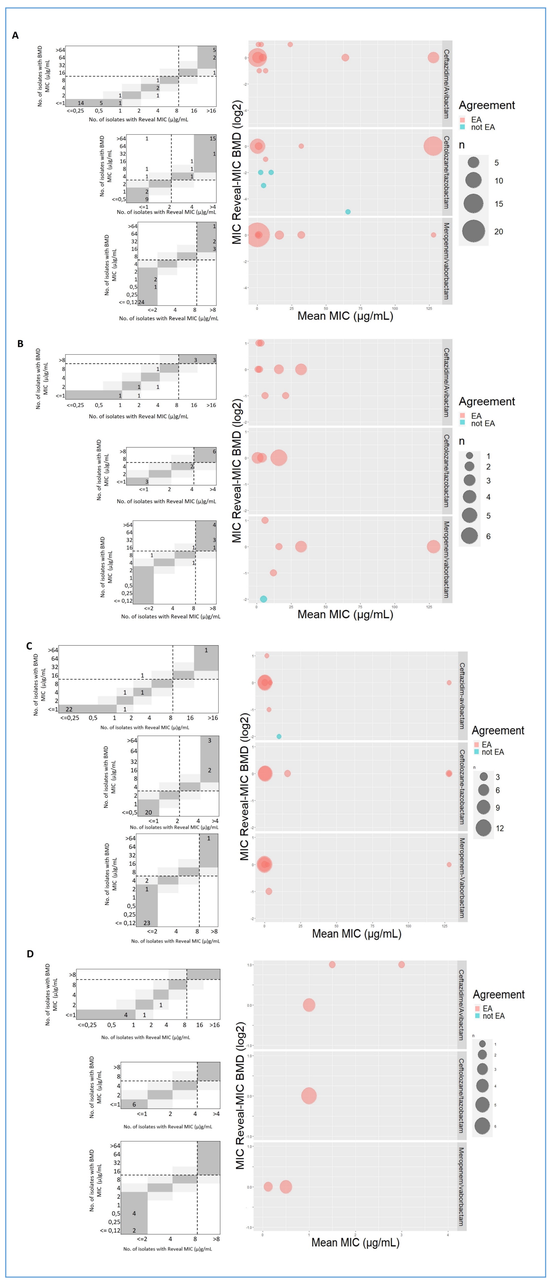


Figure 2. Performance of the VITEK® REVEAL™ AST assay for *Enterobacterales* and *Pseudomonas aeruginosa* organisms from simulated (A and B, respectively) or clinical (C and D, respectively) PBC samples tested against new β-lactam-β-lactamase inhibitor combinations.

CONCLUSIONS

The VITEK® REVEAL™ system is an excellent method for rapid AST of PBCs for antimicrobial-resistant or -susceptible bacterial pathogens. Future studies with a larger number of GN PBCs will be performed to confirm our findings.

DISCLOSURES

M. Sanguinetti and G. Menchinelli have been invited speaker in bioMérieux sponsored event.
C. Magrì and D. Squitieri PhD programs have been co-sponsored by bioMérieux.

For questions contact
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REFERENCES

- Tibbetts R, George S, Burwell R, Rajeev L, Rhodes PA, Singh P, Samuel L. Performance of the Reveal Rapid Antibiotic Susceptibility Testing System on Gram-Negative Blood Cultures at a Large Urban Hospital. J Clin Microbiol. 2022 Jun 15;60(6):e0009822. doi: 10.1128/jcm.00098-22. Epub 2022 May 24. PMID: 35607972; PMCID: PMC9199398.
- Couchot, J et al. "Evaluation of the Reveal® rapid AST system to assess the susceptibility of Pseudomonas aeruginosa from blood cultures." European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology vol. 42,3 (2023): 359-363. doi:10.1007/s10096-023-04556-2
- Menchinelli G, Liotti FM, Fiori B, De Angelis G, D'Inzeo T, Giordano L, Posteraro B, Sabbatucci M, Sanguinetti M, Spanu T. In vitro Evaluation of BACT/ALERT® VIRTUO®, BACT/ALERT 3D®, and BACTEC™ FX Automated Blood Culture Systems for Detection of Microbial Pathogens Using Simulated Human Blood Samples. Front Microbiol. 2019 Feb 19;10:221. doi: 10.3389/fmicb.2019.00221. Erratum in: Front Microbiol. 2019 Nov 22;10:2688. PMID: 30837964; PMCID: PMC6389693.
- Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 1: reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. International Organization for Standardization—ISO, Geneva, Switzerland (2019)

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