Saturday - 2070

Antibiotic Therapy Effects on Enterobacteriaceae Detection Directly from Blood: Pilot Study Implications for Future Clinical Trial Design

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Amended Abstract

Background: Detection of bacteremia directly from blood may improve time to clinical diagnosis and initiation of appropriate antibiotic therapy for hospitalized patients. Administration of empiric antibiotic therapy, whether prior to standard of care (SOC) or research study blood collection, adds to challenges in bacterial recovery. Strategies to improve detection were explored in this pilot study to inform future clinical trial design (CTD) on *Enterobacteriaceae* (ENT) detection directly from blood. The objective of this study was to assess effects of prior antibiotic administration on novel assay performance.

Methods: Confirmed ENT bacteremic (Protocol A (P-A), n=26), and suspected bacteremic (Protocol B (P-B), n=25) participants were enrolled into one of two IRB approved protocols after obtaining informed consent. Fresh whole blood (20 mL) was collected within 12 h of SOC blood culture positivity (P-A) or 20 h of SOC blood culture collection (P-B), and divided: 10 mL were inoculated into a lytic media collection vessel for the test method (P-A & B); and 10 mL into a BD BACTEC™ bottle as the control comparator (P-A). The comparator for P-B was SOC. For the test method, a 3 h amplification step in lytic growth medium followed by cleanup and concentration steps was employed. Processed samples were plated and tested using an investigational assay for universal bacterial detection on the Accelerate Pheno™ system. Results were analyzed manually and with proprietary software. Test positive samples by plating had the organism ID confirmed using the Accelerate PhenoTest™ BC kit on the Accelerate Pheno™ system. Descriptive statistics were performed to inform future CTD.

Results: Empiric antibiotic therapy was initiated prior to blood collection in 89% (P-A) and 36% (P-B) of participants. Improved detection sensitivity was achieved in P-B over P-A, when a study sample was obtained prior to empiric antibiotic therapy initiation (See Table 1).

Conclusions: Prior antibiotic administration and low bacterial load in clinical samples affects ability to detect ENT directly from blood. Multiple factors are critical to address in future CTD to increase sensitivity of detecting ENT directly from blood including: (1) Targeting study samples prior to antibiotic therapy initiation and (2) Using enzymatic methods to neutralize antibiotics present in the blood.

Methods

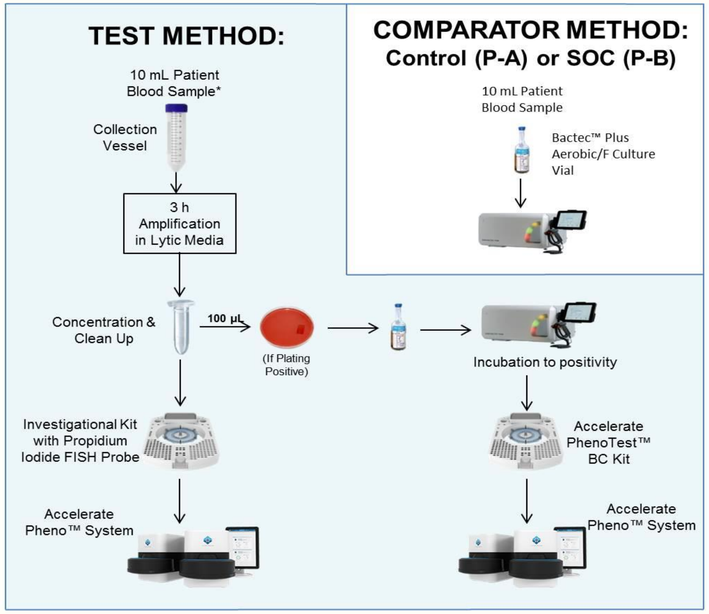
Patients were enrolled in one of two protocols:

- Confirmed *Enterobacteriaceae* (ENT) bacteremic patients were enrolled into Protocol A (P-A), n = 26
- Suspected bacteremia patients enrolled into Protocol B (P-B), n=25
- 20 mL of fresh whole blood collected after consent obtained
- Blood collected within 12 h of SOC blood culture positivity (P-A) or 20 h of SOC blood culture collection (P-B)
- Samples prepared and tested in accordance with steps outlined in *Figure 1*
- Descriptive statistics performed to inform future CTD

Research reported in this abstract and poster was supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award number R01AI116993.
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Methods

Figure 1. Protocol A & B Test and Comparator Methods



*For P-A, blood was initially added to a BD SPS® Vacutainer containing functionally the same media used in the P-B collection vessel.

Results

- Empiric antibiotic therapy initiated prior to blood collection in 89% (P-A), and 36% (P-B) participants
- Compared to P-A, improved detection sensitivity was achieved in P-B, where a larger fraction of the samples were obtained prior to empiric antibiotic therapy initiation
- Confirmed ENT bacteremic patients yielded lower sensitivity (50%) compared to suspected bacteremic patients (67%), in detecting ENT directly from blood

Results

Table 1: Comparative Descriptive Results of P-A & P-B

	P-A	P-B
Total samples	26	25
Prior ABT	89%	36%
% positive, n	15%, 5*	24%, 6
SOC Positive	N/A**	6
SOC Negative	N/A	19
Control Positive	4	N/A
Control Negative	22	N/A
Test Positive	3	4
Test Negative	23	21
Sensitivity	**50%**	**67%**
Specificity	96%	100%

*Collection vessel detected 1 positive sample that the BD BACTEC™ bottle control missed.
**SOC Positive was used as a screen for enrollment into P-A protocol, but not as a comparator.

Table 2: 2x2 Tables for P-A

P-A		Control Results: Positive (Pathogen)	Negative	Total
Test Results	Positive (Pathogen)	2*	1	3
	Negative	2	21	23
	Total	4*	22	26

*1 sample had no prior ABT

Table 3: 2x2 Tables for P-B

P-B		SOC Results: Positive (Pathogen)	Negative	Total
Test Results	Positive (Pathogen)	4*	0	4
	Negative	2	19	21
	Total	6**	19	25

*3 samples had no prior ABT
**4 samples had no prior ABT

Limitations

- Waiting for confirmation of *Enterobacteriaceae* (ENT) bacteremia in the clinical patient population prior to enrolling in a clinical trial decreases ability to detect ENT directly from blood due to an increased likelihood of effective antibiotic therapy initiation.
- For suspected bacteremic patients with empiric antimicrobial therapy initiated prior to research blood sample collection, prompt neutralization of antibiotics present in the sample in advance of direct from blood testing for ENT can be beneficial.

Conclusions

- Prior antibiotic administration and low bacterial load in clinical samples negatively impacts the ability to detect ENT directly from blood.
- Clinical trial design for increased sensitivity could include:
  1. Targeting patient enrollment prior to antibiotic therapy initiation and before SOC blood culture confirmation of ENT bacteremia
  2. Use enzymatic methods to neutralize antibiotics present in the blood.