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Effectiveness of Antibiotic Neutralization with Broad-Spectrum Beta-Lactamase in Contrived Whole Blood Samples

I. Yushkevich[1], S. Giddins[1], M. Fuchs[2], A. Irwin[1], S. Metzger[2], C. Price[1];

[1]Denver Health and Hospital Authority, Denver, CO, [2]Accelerate Diagnostics, Inc., Tucson, AZ

Irina Yushkevich
Phone: +1-303-602-2377
Irina.Yushkevich@dhha.org

Abstract

Background: *Antibiotics present in clinical blood specimens necessitate antibiotic neutralization techniques to aid in bacteremia detection. This has traditionally been addressed by utilizing dilution, charcoal, binding resins, and amine-containing compounds. In this study, broad-spectrum beta-lactamase (BSBL) was assessed as an alternative inactivating agent in contrived whole blood samples.*

Methods: *BSBL testing was performed with selected 3rd- and 4th-generation cephalosporins (ceftazidime, ceftriaxone, and cefepime), carbapenems (imipenem, meropenem, and ertapenem), and piperacillin/tazobactam at peak plasma concentrations per their respective package inserts and susceptible ATCC and CDC E. coli, K. pneumoniae, and E. aerogenes strains. Contrived whole blood samples composed of 10 mL of donor whole blood, bacterial inoculum, and an antibiotic (ABX) were added to 30 mL of lytic growth medium, protease, and 250 µL of BSBL at 66.7 U/mL. BSBL only, ABX only, and positive growth controls were run in parallel. Contrived samples were shaker incubated at 35˚C for 3 h, then plated onto blood agar. Colonies were counted after overnight growth, and recovery was calculated as a percentage of positive growth control.*

Results: *Average BSBL control and test sample recovery varied between 78-153% compared to positive growth controls, whereas all ABX controls challenged with the same ABX concentrations demonstrated no growth (Table 1).*

Table 1. Microbial Recovery Summary

Organism	Average Recovery, Percentage of Positive Growth Control, %				
	ABX Control*	BSBL Control	3rd- and 4th-Generation Cephalosporins + BSBL	Piperacillin/ Tazobactam + BSBL	Carbapenems + BSBL
E. coli ATCC® 25922™	0	99	78	97	93
E. coli CDC AR-0077	0	106	153	108	83
K. pneumoniae ATCC® 700603™	0	134	113		117
K. pneumoniae CDC AR-0016	0	123	120	92	112
E. aerogenes ATCC® 13048™	0	95	107		119
E. aerogenes CDC AR-0018	0	110	112	137	134

* all ABX controls
not applicable; resistant organism

Conclusions: *In contrived whole blood samples, BSBL effectively inactivated 3rd- and 4th-generation cephalosporins, carbapenems, and piperacillin/tazobactam, which are commonly prescribed as empiric therapies to patients with suspected gram-negative bacteremia. A clinical study is in progress including the evaluation of BSBL in patient whole blood samples.*

Introduction

- Neutralizing the effect of antibiotics in whole blood samples is included in one of the aims of the NIH grant RO1AI116993 "Ultrarapid culture-independent detection of high-priority carbapenem resistant *Enterobacteriaceae* directly from blood".
- Broad-spectrum beta-lactamase (BSBL) (AG Scientific, Inc.) was evaluated as an antibiotic neutralization agent in contrived whole blood samples.

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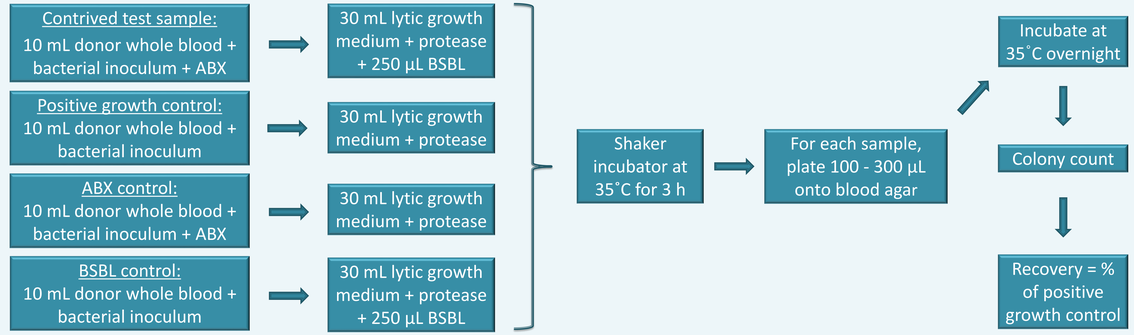
Methods

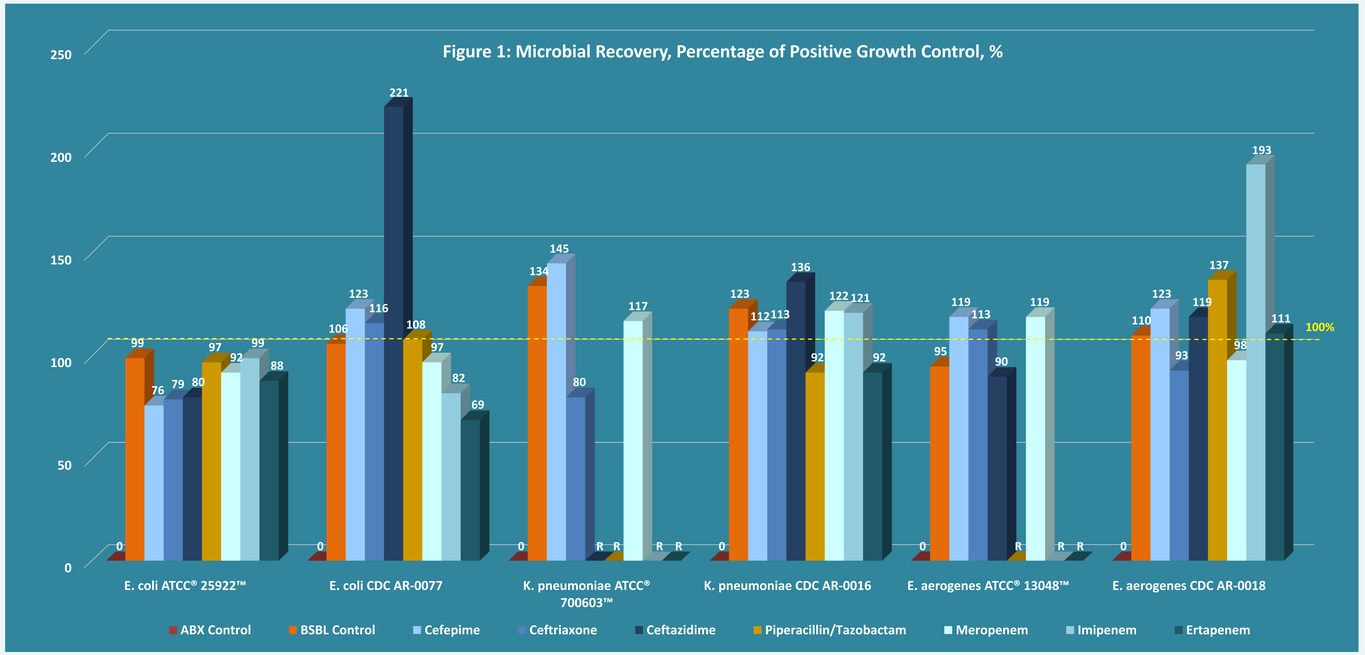
Selected 3rd- and 4th-generation cephalosporins (ceftazidime, ceftriaxone, and cefepime), carbapenems (imipenem, meropenem, and ertapenem), and piperacillin/tazobactam were tested at peak plasma concentrations per their respective package inserts. See Table 2 for ABX concentrations.

Susceptible ATCC and CDC *E. coli*, *K. pneumoniae*, and *E. aerogenes* strains were used for inoculum preparation.

Table 2: Tested Antibiotic Concentrations

Antibiotic	Peak Plasma Concentration, µg/mL
Cefepime	163
Ceftriaxone	257
Ceftazidime	170
Piperacillin/Tazobactam	298/34
Meropenem	49
Imipenem	83
Ertapenem	283





Results

- Starting sample concentrations were 16-36 CFU/mL.
- After 3 h incubation, final sample concentrations of positive growth controls were 1-7x10^4 CFU/mL.
- There was no growth in all ABX controls.
- BSBL controls demonstrated 95-134% recovery compared to positive growth controls. No enzyme toxicity was observed.
- Average test sample recovery varied between 78-153% compared to positive growth controls. See Figure 1 for microbial recovery for all organism/ABX combinations. "R" indicates that the organism is resistant to the antibiotic and the combination was not included in the evaluation.

Limitations

Per manufacturer description, BSBL is only effective against penicillins, cephalosporins, and carbapenems.

Conclusions

BSBL effectively inactivated 3rd- and 4th-generation cephalosporins, carbapenems, and piperacillin/tazobactam in contrived whole blood samples. A clinical study is in progress including the evaluation of BSBL in patient whole blood samples.