In vitro Synergy of Ceftazidime-Avibactam Plus Rifampin Against *Pseudomonas aeruginosa*

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Revised Abstract

Background:
Pseudomonas aeruginosa is a major cause of nosocomial infections. An emerging concern is the increased prevalence of multidrug-resistance limiting treatment options. Ceftazidime-avibactam (CZA), FDA-approved February 2015, is a novel cephalosporin/non-β-lactam β-lactamase inhibitor coupling that demonstrates antipseudomonal activity. Despite its recent release, resistance to this agent has been reported. Rifampin (RI) has shown *in vitro* and *in vivo* synergistic activity when combined with β-lactams and polymyxins against *P. aeruginosa*. The aim of this study was to investigate any antimicrobial synergistic activity of the combination of CZA and RI against CZA-R *P. aeruginosa* isolates.
Methods:
P. aeruginosa isolates were prospectively collected from 193 consecutive patients during the last five months of 2015. Recovery sources were respiratory (45%), urinary (24%), wound (16%), bloodstream (9%), and other (6%) infections. Initial antimicrobial susceptibilities were determined by MicroScan. CZA and RI MICs (μg/mL) were determined by Etest. FDA MIC (μg/mL) interpretive guidelines *for* CZA are ≤8 susceptible, ≥16 resistant. There are no CLSI or FDA interpretive guidelines for testing RI against *P. aeruginosa*. Rep-PCR analysis was used to determine that CZA-R isolates were genetically unique. MICs and synergy testing (MIC: MIC method) of CZA-R isolates were performed in triplicate. The summation fractional inhibitory concentration (∑FIC) was calculated: synergy, ≤0.5; additivity, >0.5-1.
Results:
9/193 (5%) *P. aeruginosa* isolates were resistant to CZA, including 6/27 (22%) with multidrug-resistance (resistant to ≥1 agent in ≥3 antimicrobial categories). *In vitro* synergy by Etest was detected in 6/9 (67%) isolates (∑FICs 0.1-0.5) and additivity, 3 (∑FIC 0.9-1.0).
Conclusion:
Even though ceftazidime-avibactam demonstrated excellent activity in the *P. aeruginosa* isolates tested, resistance has already occurred. However, *in vitro* synergy or additivity was demonstrated against these CZA-R isolates with CZA plus rifampin. This combination should be further tested with additional isolates. *In vitro* synergy may not predict *in vivo* response.

Introduction

Pseudomonas aeruginosa is a major cause of nosocomial infections. An emerging concern is the increased prevalence of multidrug-resistance limiting treatment options. Ceftazidime-avibactam (CZA) is a novel cephalosporin/non-β-lactam β-lactamase inhibitor coupling that demonstrates antipseudomonal activity. Resistance to this agent has been reported (Winkler et al. 2015 *AAC* 59:1020-9). Rifampin (RI) has shown *in vitro* and *in vivo* synergistic activity when combined with β-lactams and polymyxins against *P. aeruginosa* (Mitsugui et al. 2011 *Int J Antimicrob Agents* 38:447-50). The aim of this study was to investigate any *in vitro* antimicrobial synergistic activity of the combination of CZA and RI against CZA-R *P. aeruginosa* isolates.

Methods

Bacterial Isolates: *P. aeruginosa* isolates were prospectively collected from 193 consecutive patients during the last five months of 2015. Recovery sources were respiratory (45%), urinary (24%), wound (16%), bloodstream (9%), and other (6%) infections. Rep-PCR analysis (Diversilab, bioMérieux) was used to determine that CZA-R isolates were genetically unique. Multidrug-resistance was defined as resistant to ≥ 1 agents in ≥ 3 antimicrobial categories.

Media and Controls: Mueller-Hinton II broth and agar plates (Becton-Dickinson) were used for Etest MICs and synergy tests. *P. aeruginosa* ATCC 27853 was used as the control strain (2016 CLSI).

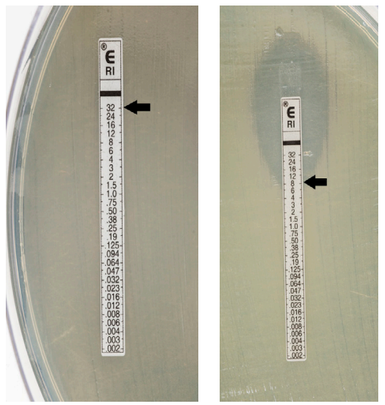

Figure 1. Isolate #4
Left: Rifampin (RI) original MIC >32 μg/mL
Right: RI after combination with ceftazidime-avibactam (at 12 μg/mL)
MIC = 8 μg/mL.
FIC RI = 0.13

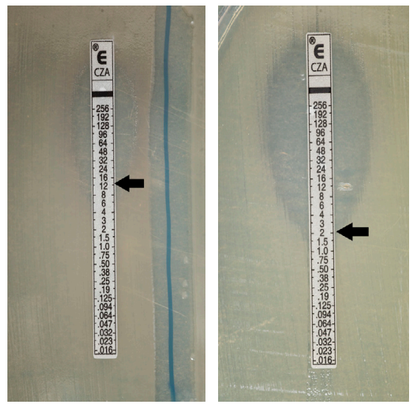

Figure 2. Isolate #4
Left: Ceftazidime-avibactam (CZA) original MIC = 12 μg/mL
Right: CZA after combination with rifampin (at 32 μg/mL)
MIC = 2 μg/mL.
FIC CZA = 0.17

ΣFIC = 0.13 + 0.17 = 0.3 (*synergy*)

MIC determination and Synergy testing: CZA and RI MICs (μg/mL) were determined by Etest following manufacturer's guidelines (bioMérieux). Etest synergy testing (MIC:MIC method) (Fig.1,2) of CZA-R isolates was performed in triplicate, mean values were used (Pankey et al 2005 *AAC* 49:2959-64). Plates were inoculated with suspensions of isolates. Etest strips for each antimicrobial were added to the bacterial lawn sequentially, and the strips were marked at the MIC and removed after 1 h incubation at room temperature. The second strips were immediately added over the imprint of the first strips aligning the MICs of each strip. The plates were incubated for 16-20 h at 35°C. MICs were read at the point of complete inhibition. The following formulas were used to determine summation fractional inhibitory concentration (∑FIC):

FIC RI = MIC of RI in combination ÷ MIC of RI alone

FIC CZA = MIC of CZA in combination ÷ MIC of CZA alone

ΣFIC = FIC RI + FIC CZA.

Synergy was determined at ∑FIC ≤0.5; additivity, ∑FIC >0.5-1.

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Results (Table)

- 184/193 (95%) *P. aeruginosa* isolates were susceptible to CZA, including 21/27 (78%) multi-drug resistant isolates.
- 6/27 (22%) multi-drug resistant isolates were resistant to CZA.
  With CZA + RI:
  - 5/6 (83%) displayed synergy (ΣFICs 0.2-0.5)
  - 1/6 (17%) displayed additivity (ΣFIC 1.0)
- 3/9 *P. aeruginosa* isolates (3,5,9 in Table) were not multi-drug resistant, but were resistant to CZA. Synergy was detected in 1 isolate and additivity, in 2 isolates.
- In total, 9/193 (5%) *P. aeruginosa* isolates were resistant to CZA.
  With CZA + RI:
  - *In vitro* synergy was detected in 6/9 (67%) isolates (ΣFICs 0.1-0.5).
  - *In vitro* additivity was detected in 3/9 (33%) isolates (ΣFICs 0.9-1.0).

MICs (μg/mL) and Synergy Testing with CZA and RI against Ceftazidime-avibactam resistant (CZA-R) *P. aeruginosa* Isolates determined by Etest.

CZA–R *P. aeruginosa* Isolates	Rifampin (RI) original MIC*	Ceftazidime-avibactam (CZA) original MIC*	Etest Synergy Method (CZA + RI) (1 x MIC) ΣFIC*	
# 1 (MDR)	>32	24	1.0	ADD
# 2 (MDR)	>32	>256	0.2	SYN
# 3	>32	128	0.1	SYN
# 4 (MDR)	>32	12	0.4	SYN
# 5	>32	12	0.9	ADD
# 6 (MDR)	>32	8	0.5	SYN
# 7 (MDR)	>32	24	0.4	SYN
# 8 (MDR)	>32	>256	0.3	SYN
# 9	>32	12	0.9	ADD

MDR, multidrug-resistant.
FDA MIC (μg/mL) interpretive guidelines for CZA (*P. aeruginosa*) are ≤8 susceptible, ≥16 resistant.
There are no CLSI or FDA interpretive guidelines for testing RI against *P. aeruginosa*.
*Performed in triplicate (mean value used); ADD = Additivity; SYN = Synergy

Conclusion

- Even though ceftazidime-avibactam demonstrated excellent activity against *P. aeruginosa* isolates tested, resistance has already occurred.
- Ceftazidime-avibactam resistant *P. aeruginosa* isolates were inhibited *in vitro* by ceftazidime-avibactam plus rifampin (synergy, 6; additivity, 3).
- Ceftazidime-avibactam plus rifampin should be further tested with additional ceftazidime-avibactam resistant *P. aeruginosa* isolates.
- *In vitro* synergy may or may not predict *in vivo* response.