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# Imipenem/Cilastatin/Relebactam (I/R) Alone and in Combination against Multidrug-Resistant (MDR) *Pseudomonas aeruginosa* (PSA) in the *In Vitro* Pharmacodynamic Model (IVPD)

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## ABSTRACT (Revised)

**Background:** I/R, a carbapenem-beta-lactamase inhibitor antibiotic, is active against most imipenem-resistant PSA, including MDR isolates. Combination therapy may enhance activity against MDR pathogens and suppress resistance. This study's objective was to assess the efficacy of I/R compared with combinations including colistin (CST) or amikacin (AMK) against PSA in an IVPD model.

**Methods:** Human-simulated concentrations of I/R 500/250 mg every six hours, a total daily dose of CST 360 mg, and AMK 25 mg/kg daily were reproduced alone and in combination against 6 imipenem-non-susceptible PSA with I/R MICs of 1/4 to 8/4 mg/L in an IVPD over 24h. The primary endpoint was the difference in 24h colony forming units (CFU) between each combination regimen and its components alone. The log ratio differences of the area under the CFU curve were also calculated to compare the overall bacterial burden resulting from exposure to I/R alone with those treated by combination regimens. Emergence of resistance was tested at 24h using drug-containing plates at 3xMIC.

**Results:** I/R, CST, and AMK alone produced 24hCFU changes consistent with isolate MICs. One isolate (already I/R non-susceptible) developed I/R resistance, and 4 and 3 developed CST and AMK resistance, respectively. I/R plus CST suppressed all resistance and resulted in synergistic or additive interactions against three of six isolates with 24h CFU reductions ranging from -2.62 to -4.67 $\log_{10}$CFU/mL. This combination further reduced overall bacterial burden by 79-81% compared with I/R alone against two I/R-non-susceptible strains. I/R plus AMK also prevented resistance emergence but exhibited indifferent interactions against all isolates at 24h with the combined drugs achieving -0.51 to -3.33 $\log_{10}$CFU/mL reductions. Minor overall reductions in bacterial burden were observed relative to I/R alone.

**Conclusions:** I/R plus CST resulted in additivity or synergy against three of six PSA and prevented I/R and CST resistance, whereas the addition of AMK only suppressed resistance. The greatest overall reductions in bacterial burden, however, were observed with I/R plus CST against I/R-non-susceptible isolates, supporting targeted use of this combination against this phenotype when alternatives are unavailable.

## INTRODUCTION

- The diminishing susceptibility of *Pseudomonas aeruginosa* to carbapenems, the last-line agents for multidrug-resistant (MDR) strains, highlight the need for new therapies (1,2)
- Imipenem/cilastatin/relebactam (I/R) is a β-lactam/β-lactamase inhibitor combination antibiotic (3)
  - Relebactam (REL) restores imipenem activity against *P. aeruginosa*, potentiating its minimum inhibitory concentration (MIC) by up to eightfold (4)
- Despite the potency of I/R, treatment failure and resistance emergence remains a concern and many clinicians consider combination therapy for severe *P. aeruginosa* infections (5)
- Colistin (CST) and aminoglycosides are potential add-on agents given their differing mechanisms of action and lower resistance rates in most institutions (5-7)

## OBJECTIVE

To compare the antibacterial activity of I/R alone and combined with CST or amikacin (AMK) against six imipenem-non-susceptible *P. aeruginosa* isolates in the *in vitro* pharmacodynamic model.

## METHODS

### *Study Isolates*

- Six imipenem non-susceptible *P. aeruginosa* isolates were selected
- Modal MICs of I/R, imipenem (IPM), CST, and AMK were determined in triplicate per Clinical Laboratory Standards Institute (CLSI) guidelines (8). Ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (CZA) MICs of the CAIRD isolates were obtained in this manner previously (data on file). Those of the CDC isolates were obtained from the CDC/FDA AR Bank (www.cdc.gov/drugresistance/resistance-bank/index.html).

### *Antibacterial Agents*

- Agents
  - IPM (Lot 0000685746) (Merck & Co., Inc.; Kenilworth, NJ)
  - REL (Lot 002D039) (Merck & Co., Inc.; Kenilworth, NJ)
  - CST (Lot SLCB7174) (Sigma-Aldrich; St. Louis, MO)
  - AMK (Lot 115837/B) (Medisca; Plattsburgh, NY)
- Regimens were designed to simulate human exposures of
  - I/R 500/250 mg q6h as 0.5 hour infusions (9)
  - A total daily dose of CST 360 mg (10)
  - AMK 1750 mg (25 mg/kg in a 70 kg patient) q24h (11)

### In vitro *Pharmacodynamic Chemostat Model*

- Each bug-drug combination experiment used two treatment reactors and one antibiotic-free control reactor
- Each reactor contained 150 mL of cation-adjusted Mueller-Hinton broth and a magnetic stir bar, and were kept in a 35° C water bath
- Reactors were inoculated with bacterial suspensions prepared to a target of $10^6$ $\log_{10}$CFU/mL
- Thirty minutes after inoculation (0h), treatment was initiated and sterile broth was infused through a peristaltic pump to target the I/R $t_{1/2}$.
- All experiments ran over 24 hours following antibiotic administration

### *Antibacterial Activity*

- The antibiotic activity of each regimen was determined by the difference in $\log_{10}$CFU/mL difference observed between 0 and 24 hours compared with the most active applicable monotherapy (12-14)
  - Synergy: >2-log10CFU/mL difference
  - Additivity: 1- to 2-log10CFU/mL difference
  - Indifference : <1-log10CFU/mL difference
- The log ratio(LR) difference in area under the CFU curve (AUCFU) was determined to analyze the overall change in bacterial burden between treatment regimens (12)

### *Resistance Determination*

- The development of resistance was monitored at 24 hours
- Samples were plated on I/R, colistin, or AMK-containing agar plates prepared prior to the experiments. The concentrations were dependent on the isolate and were targeted to 3x MIC.
  - REL concentrations were kept at 4 mg/L in all I/R plates
  - CST plates were prepared to 3 mg/L to be above the *P. aeruginosa* breakpoint of 2 mg/L. (8)
- Plates were incubated at 37 °C for 18 to 24 hours.
- Resistance was present when the colony count on the plate was greater than the lower limit of detection (LLD) of 1.7 $\log_{10}$ CFU/mL

### *Antibiotic Concentrations and Exposures*

- Assays
  - IPM/REL: high performance liquid chromatography
  - CST: liquid chromatography-tandem mass spectrometry
  - AMK: enzyme multiple-immunoassay

## RESULTS

### *In Vitro Susceptibility*

**Table 1** Modal MICs of the six study isolates

| Isolate | Antibiotic MIC (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | I/R | IPM | CST | AMK | C/T | CZA |
| CAIRD M8-29 | 1/4 | 4 | 1 | 8 | 1/4 | 8/4 |
| CDC0270 | 2/4 | 16 | 0.5 | 16 | N/A[a] | N/A[a] |
| CDC0526 | 2/4 | 16 | 1 | 8 | 2/4 | 2/4 |
| CDC0527 | 4/4 | 32 | 0.5 | 0.5 | 1/4 | 8/4 |
| CAIRD M23-3 | 4/4 | 32 | 1 | 32 | 2/4 | 4/4 |
| CAIRD M1-4 | 8/4 | 32 | 0.5 | 32 | 4/4 | 8/4 |

[a] N/A, Not available

### *Human-simulated Exposures*

- All target concentrations and exposures were achieved
- Observed versus targeted concentrations and exposures are displayed in Table 2

**Table 2** Observed versus predicted concentrations and pharmacodynamic exposures[a]

| Drug | $fC_{max}$ (mg/L) | | $t_{1/2}$ (h) | | %fT > MIC | | $fAUC_{0-24}$ (mg*h/L) | | fAUC/MIC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Target | Observed | Target | Observed | Target | Observed | Target | Observed | Target | Observed |
| IPM | 25 | 24.7 (22.7-28.6) | 1.2 | 1.1 (1.1-1.2) | 70 | 69 (64-75) | NA | NA | NA | NA |
| REL | 13 | 15.1 (13.9-18.0) | 1.2 | 1.2 (1.1-1.2) | NA | NA | 84 | 108 (97-125) | 21 | 27 (24-31) |
| CST | 0.73 | 0.85 (0.79-0.91) | NA | NA | NA | NA | NA | NA | NA | NA |
| AMK | NA | NA | NA | NA | NA | NA | 348 | 377 (350-410) | 22 | 23 (22-25) |

[a] Data are reported as the median (interquartile range, IQR). $fC_{max}$, free maximum concentration; $t_{1/2}$, half-life; % *f*T>MIC, percent free time above the minimum inhibitory concentration; $fAUC_{0-24}$, area under the curve over the 24-h experiment; NA, not applicable.
[b] Calculated using an imipenem/cilastatin/relebactam MIC of 2/4 mg/L.
[c] Due to continuous infusion in the model, colistin $fC_{ss}$ is the mean concentration observed over the 24 hour experiment.
[d] Calculated using an amikacin MIC of 16 mg/L.

### *Antibacterial Efficacy*

- The results of the pharmacodynamic studies are depicted in Figures 1 and 2
  - I/R+CST demonstrated synergy against one isolate (CDC0526) and additivity against two isolates (CAIRD M8-29 and CAIRD M23-3)
  - I/R+AMK demonstrated indifference against all studied isolates
- The LR for AUCFU data are listed in Table 3
  - The greatest decreases with combination therapy relative to I/R alone were observed with I/R+CST against CAIRD M23-3 (-0.68 log or 79% reduction) and CAIRD M1-4 (-0.71 log or 81% reduction)

**Table 3** LR of AUCFU for antibiotic monotherapy compared with control and for combination regimens

| Isolate | Log difference in AUCFU | | | | | | |
|---|---|---|---|---|---|---|---|
| | I/R / control | CST / control | AMK / control | I/R+CST / I/R alone | I/R+CST / CST alone | I/R+AMK / I/R alone | I/R+AMK / AMK alone |
| CAIRD M8-29 | -2.80 | -2.67 | -2.06 | 0.10 | 0.11 | 0.14 | -0.55 |
| CDC0270 | -2.76 | -2.72 | -1.35 | -0.08 | -0.14 | 0.42 | -1.11 |
| CDC0526 | -2.54 | -2.65 | -2.38 | -0.30 | -0.19 | -0.11 | -0.09 |
| CDC0527 | -2.92 | -2.87 | -2.89 | -0.09 | 0.04 | -0.20 | -0.02 |
| CAIRD M23-3 | -2.32 | -2.62 | -0.49 | -0.68 | -0.33 | 0.15 | -1.48 |
| CAIRD M1-4 | -1.96 | -2.82 | -0.54 | -0.71 | 0.10 | -0.15 | -1.75 |

### *Resistance*

- After 24 hours of exposure to each agent alone, resistance developed to
  - I/R in one isolate (CAIRD M23-3)
  - CST in four isolates (CAIRD M8-29, CDC0270, CDC0526, and CAIRD M23-3)
  - AMK in three isolates (CAIRD M8-29, CDC0270, and CAIRD M1-4)
- I/R+CST combination therapy suppressed I/R and CST resistance in all applicable isolates
- I/R+AMK combination therapy suppressed AMK resistance in all applicable isolates

**Figure 1** Mean number of CFU over 24h by isolate. Data are presented as the mean CFU from experiment replicates of each regimen. Sold line/no symbol, control; dashed line/no symbol, I/R alone; solid line/square, colistin alone; solid line/triangle, amikacin alone; dashed line/square, I/R plus colistin combination therapy; dashed line/triangle, I/R plus amikacin combination therapy; dotted line, lowest limit of detection (LLOD).

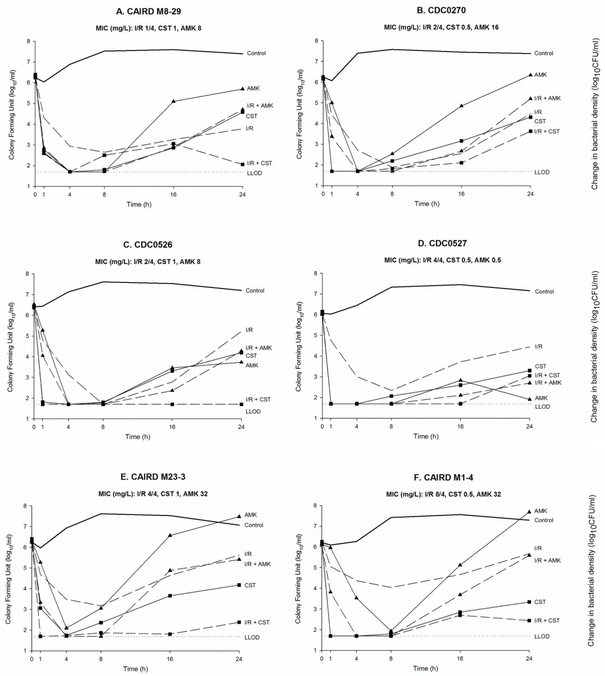
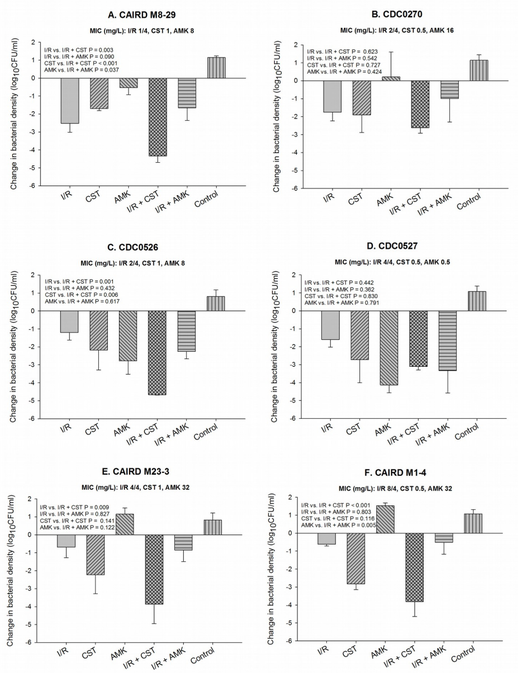



**Figure 2** Mean change at 24h in bacterial density from 0h. All treatments were significantly different from their controls ($p < 0.05$) except for I/R against CAIRD M23-3 and AMK against CDC0270, CAIRD M23-3, and CAIRD M1-4. Statistical results between combination therapies and their constituent agents alone are reported in the upper left corner of each graph.




## CONCLUSIONS

- **I/R+CST combination therapy improved treatment efficacy against three of six IPM-non-susceptible *P. aeruginosa* isolates and suppressed resistance to both agents in all applicable isolates**
- **I/R+AMK combination therapy did not further reduce bacterial burden, but did suppress AMK resistance in all applicable isolates**
- **The greatest overall reductions in bacterial burden occurred when I/R plus CST was used against I/R-non-susceptible isolates**
- **These results support the targeted use of I/R plus CST against I/R-non-susceptible *P. aeruginosa* isolates when alternatives are unavailable**

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