

# THE LANCET Microbe

## Supplementary appendix

This appendix formed part of the original submission. We post it as supplied by the authors.

Supplement to: Choby JE, Ozturk T, Satola SW, Jacob JT, Weiss DS. Does cefiderocol heteroresistance explain the discrepancy between the APEKS-NP and CREDIBLE-CR clinical trial results?. *Lancet Microbe* 2021; published online Oct 28. [https://doi.org/10.1016/S2666-5247\(21\)00271-8](https://doi.org/10.1016/S2666-5247(21)00271-8).

## **METHODS**

### **Isolate information**

Unique patient isolates of carbapenem-susceptible *Acinetobacter* spp. (from 2018-2021), *Klebsiella* spp. (2019-2020), and *Pseudomonas aeruginosa* (2018-2021) from Georgia, USA were collected as part of the Emory Antibiotic Resistance Center's Investigational Clinical Microbiology Core repository (<https://www.cores.emory.edu/icmc/>).

### **Population analysis profile**

Population analysis profile (PAP) was performed as described previously<sup>1,2</sup>. A given clinical isolate was grown overnight from a single colony isolated from frozen stock in 1.5 mL iron-depleted cation-adjusted Mueller Hinton Broth (ID-CA-MHB) prepared according to accepted protocol<sup>3</sup> at 37°C with shaking for aeration. The culture was then serially diluted and plated on Mueller-Hinton agar containing 0, 2, 4, 8, 16, or 32 µg/mL cefiderocol (Hardy Diagnostics); 16 µg/mL is the CLSI breakpoint for all three species<sup>4</sup>. Colonies were enumerated after 24-48 h of growth at 37°C. An isolate was classified as resistant if the number of colonies that grew at the breakpoint concentration was at least 50% of those that grew on antibiotic free plates. If the isolate was not resistant, it was classified as heteroresistant if the number of colonies that grew at 2 or 4 times the breakpoint were at least 0.0001% (1 in 10<sup>6</sup>) of those that grew on antibiotic free plates. If the isolate was neither classified as resistant nor heteroresistant, it was classified as susceptible.

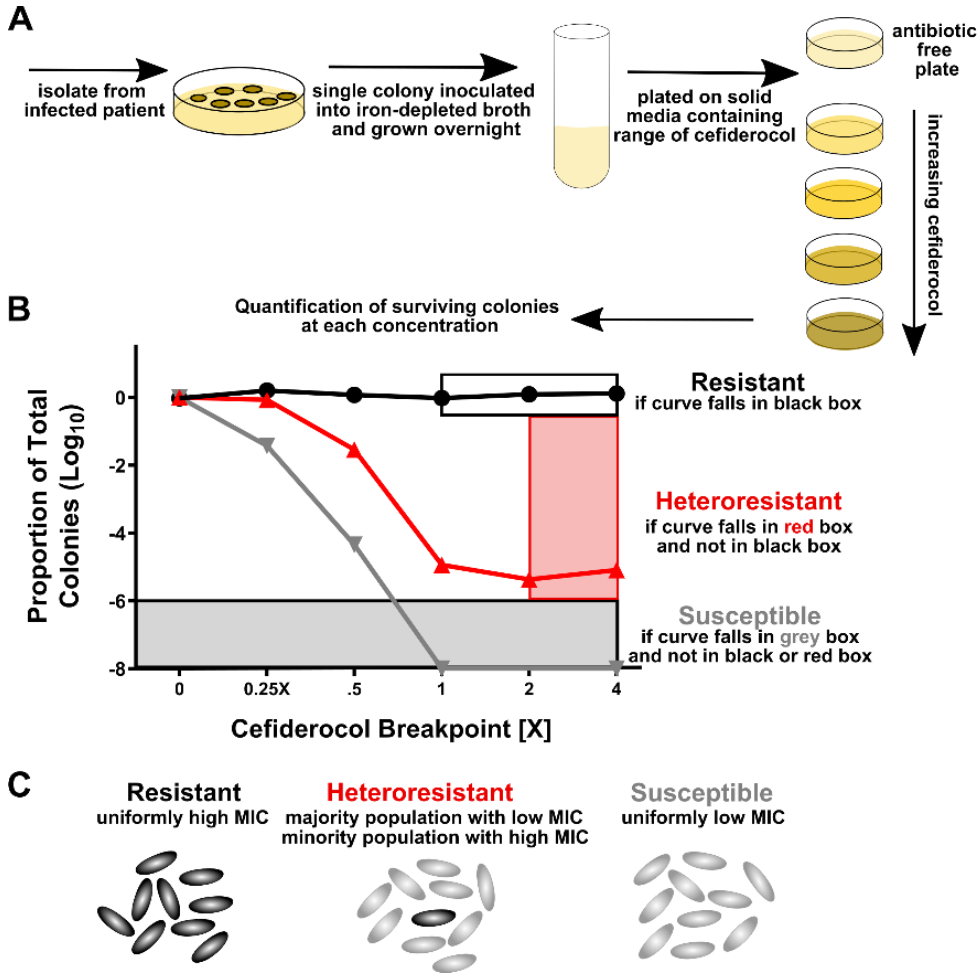
### **Cefiderocol treatment case reports**

An incomplete list of case reports in which cefiderocol was used for treatment of infections caused by carbapenem-resistant and/or multidrug resistant isolates is included as references 5 through 13.

## **ACKNOWLEDGEMENTS**

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**FIGURES AND TABLES**



**Supplemental Figure 1. Overview of heteroresistance and population analysis profile.** (A) A clinical isolate is isolated and grown in liquid medium, and then serially diluted and grown on increasing concentration of cefiderocol. (B) The surviving colonies are enumerated and the isolate is classified as resistant if at least 50% of the total colonies grow at 1, 2 or 4X breakpoints. An isolate is considered susceptible if less than -6 logs (0.0001%) grow at 1, 2 and 4X. An isolate is considered heteroresistant if there is less than 50% survival at 1X breakpoint and greater than 0.0001% at 2 or 4X breakpoint. (C) A schematic depiction of the cells grown from a single colony of an isolate.

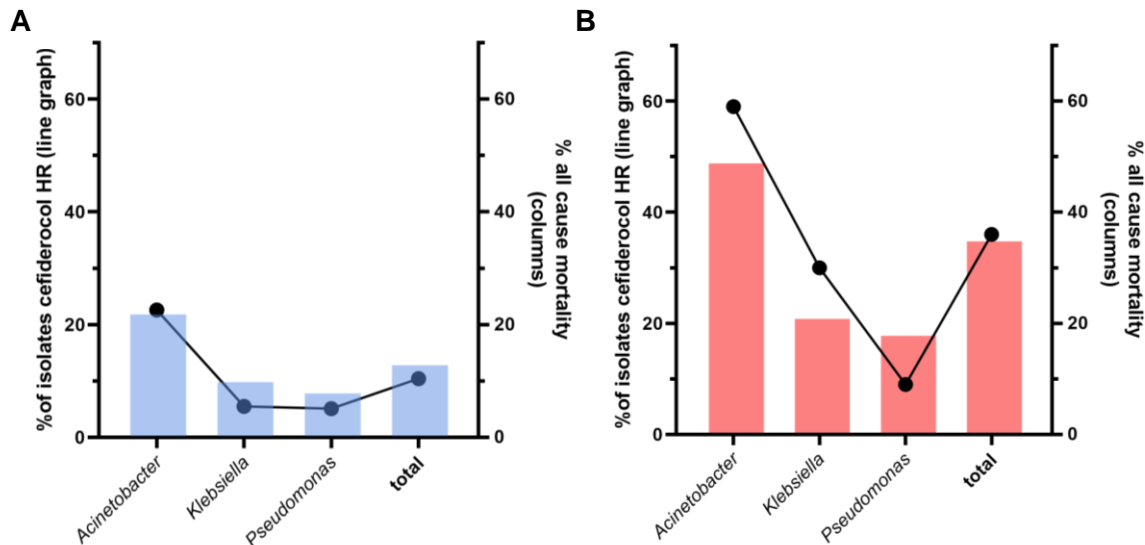
		<i>Acinetobacter</i>	<i>Klebsiella</i>	<i>Pseudomonas</i>	Total
Hetero-resistance GA, USA	Susceptible (S) <sup>A</sup>	11% [4/35]	0% [0/50]	5% [1/20]	5% [5/105]
	Cephalosporin-resistant <sup>B</sup>	44% [8/18]	12% [5/41]	5% [1/19]	18% [14/78]
	Carbapenem-resistant (CR) <sup>2</sup>	59% [64/108]	30% [27/89]	9% [6/69]	36% [97/266]
All-cause mortality	APEKS-NP <sup>14,C</sup>	22% [5/23]	10% [5/48]	8% [2/24]	13% [12/95]
	CREDIBLE-CR <sup>15,C</sup>	49% [19/39]	21% [6/28]	18% [2/11]	35% [27/78]

<sup>A</sup>These isolates are susceptible to meropenem, cefepime, ceftazidime, and ceftriaxone by clinical antimicrobial susceptibility testing.

<sup>B</sup>These isolates are carbapenem (meropenem) susceptible but resistant to at least one of the extended spectrum cephalosporins cefepime, ceftazidime, and ceftriaxone by clinical antimicrobial susceptibility testing.

<sup>C</sup>APEKS-NP involved only pneumonia patients while CREDIBLE-CR enrolled patients with pneumonia, bloodstream infections or urinary tract infections.

**Table 1. The higher rate of all-cause mortality in the CREDIBLE-CR trial versus the APEKS-NP trial correlates with the increased frequency of ceftiderocol heteroresistance among carbapenem-resistant relative to carbapenem-susceptible Gram-negative pathogens.** Heteroresistance was determined by population analysis profile (PAP) to detect minority resistant subpopulations within an isolate. Isolates were classified as susceptible (S), cephalosporin-resistant and carbapenem-susceptible, or carbapenem resistant (CR) by conventional antimicrobial susceptibility testing. Ceftiderocol heteroresistance rates among CR isolates were previously reported<sup>2</sup>. All-cause mortality data are from APEKS-NP<sup>14</sup> or CREDIBLE-CR<sup>15</sup>.



**Supplemental Figure 2. Graphical representation of the correlation between heteroresistance and all-cause mortality in the CREDIBLE-CR trial versus the APEKS-NP trial.** The left axis and line graphs show the heteroresistance rates and the right axis and columns show the all-cause mortality rate for (A) carbapenem-susceptible isolates and the APEKS-NP trial and (B) carbapenem-resistant isolates and the CREDIBLE-CR trial.

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