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LETTER TO THE EDITOR

Effect of Meropenem Concentration on the Detection of Low Numbers of Carbapenem-Resistant *Enterobacteriaceae*

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The nosocomial spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is a serious concern for health care facilities (1, 2), and environmental sources are being increasingly implicated (3–6). Carbapenems can be used to select for CRE in environmental testing, but detection may be impacted when organisms are present in low numbers. Bacterial tolerance to carbapenems can increase as inoculum size increases (7, 8), but the impact of carbapenems on low inoculum sizes is not well studied. Here we report the effect of different meropenem concentrations on the detection of low numbers of CRE.

We tested 16 NDM- or KPC-producing *Enterobacteriaceae* strains on Mueller-Hinton agar (MHA) with various concentrations of meropenem (Table 1). Concentrations of 0.5, 1.0, 2.5, 5.0, or 10.0 μg/ml were tested based on the concentrations used in other environmental CRE studies (9–11). MHA plates without antibiotic were used for comparison (control plates). The meropenem MIC for each isolate was determined with Etest strips (bioMérieux Clinical Diagnostics, Marcy l'Etoile, France). Clinical and Laboratory Standards Institute (CLSI) guidelines were followed for antibiotic quality control (12).

Log-phase cultures of each organism were serially diluted to inoculate triplicate plates with 50 to 150 organisms per plate. Colony counts were averaged for each triplicate set after overnight incubation. Percent recovery was calculated as the average colony count of the triplicate plates for each organism and meropenem concentration divided by the average colony count of the corresponding control plates. The statistical significance ($P < 0.05$) of average CFU counts on control versus meropenem plates was assessed using a two-tailed Student *t* test.

All but one of the 16 isolates (KPC-R-2) could be detected at the lowest meropenem concentration (0.5 μg/ml) (Table 1). A second KPC producer (KPC-CO-1) was detected at 0.5 μg/ml meropenem, but with less than 50% recovery ($P = 0.016$). At 1 μg/ml, two additional isolates, NDM-CO-4 and KPC-CO-4, had reduced CFU counts (recovery = 53% and 30%, respectively). At 2.5 μg/ml meropenem, 12 isolates had significantly reduced CFU counts. Only three isolates (KPC-CO-2, KPC-R-1, and NDM-CO-5) were detected at 10 μg/ml meropenem, and two had significantly reduced counts.

Inocula used to determine CLSI susceptibility breakpoints and MICs range from 10^4 cells per spot (agar dilution) to 5×10^5 cells per milliliter (broth microdilution) (13); however, in the environ-

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TABLE 1 MIC and average recoveries of organisms by meropenem concentration[b]

Organism ID	Meropenem MIC (μg/ml)[a]	Avg no. of CFU on MHA with no antibiotics	% recovery (*P* value) with indicated concn (μg/ml) of meropenem				
			0.5	1	2.5	5	10
ATCC BAA-2146 (NDM)	>32	83	123 (0.245)	113 (0.322)	96 (0.745)	6 (0.008)	0 (0.008)
NDM-CO-1	>32	121	98 (0.880)	99 (0.911)	3 (0.001)	0 (0.002)	0 (0.002)
NDM-CO-2	>32	92	112 (0.150)	110 (0.140)	96 (0.606)	3 (0.001)	0 (0.002)
NDM-CO-3	>32	100	111 (0.319)	84 (0.210)	2 (0.006)	0 (0.007)	0 (0.007)
NDM-CO-4	>32	104	96 (0.694)	53 (0.017)	0 (0.005)	0 (0.005)	0 (0.005)
NDM-CO-5	>32	78	103 (0.818)	116 (0.222)	125 (0.162)	120 (0.120)	123 (0.088)
Env. KP NDM	>32	134	98 (0.648)	100 (0.940)	68 (0.005)	0 (0.002)	0 (0.002)
Env. EC NDM	24	101	91 (0.185)	84 (0.129)	1 (0.001)	0 (0.001)	0 (0.001)
Utah NDM	>32	68	98 (0.605)	91 (0.170)	0 (<0.001)	0 (<0.001)	0 (<0.001)
KPC-R-1	>32	52	103 (0.625)	97 (0.808)	106 (0.552)	69 (0.372)	76 (0.036)
KPC-R-2	8	53	0 (0.012)	0 (0.012)	0 (0.012)	0 (0.012)	0 (0.012)
KPC-CO-1	8	53	42 (0.016)	0 (0.010)	0 (0.010)	0 (0.010)	0 (0.010)
KPC-CO-2	>32	67	120 (0.086)	103 (0.818)	11 (<0.001)	20 (<0.001)	2 (0.001)
KPC-CO-3	12	71	88 (0.140)	92 (0.278)	40 (0.030)	8 (0.001)	0 (0.003)
KPC-CO-4	16	66	85 (0.320)	30 (0.049)	0 (0.011)	0 (0.011)	0 (0.011)
KPC-CO-5	16	74	105 (0.584)	99 (0.933)	9 (0.007)	16 (0.003)	0 (0.006)

[a] MICs were determined by the meropenem Etest per the manufacturer's instructions.
[b] ID, identifier; MHA, Mueller-Hinton agar; Env., environmental.

ment, bacteria are typically present in lower numbers. In this study, recovery was significantly reduced when plates were inoculated with approximately 100 cells, even when meropenem concentrations were much lower than the organism MIC. Testing other reduced inoculum sizes would better depict the effect of meropenem on the recovery of low numbers of organisms, but these results are limited to only one inoculum size. Recovery in liquid media was not evaluated as part of this study but is described elsewhere for three isolates (ATCC BAA-2146, KPC-R-1, KPC-R-2) (14). We did not evaluate the recovery of CRE in actual or simulated environments, which might also demonstrate the impact of meropenem on selectivity. Carbapenems may effectively suppress nontarget growth in CRE environmental studies; however, high carbapenem concentrations may also suppress CRE growth, leading to inaccurate conclusions about CRE prevalence in the environment.

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