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Does the Activity of the Combination of Imipenem and Colistin In Vitro Exceed the Problem of Resistance in Metallo-β-Lactamase-Producing *Klebsiella pneumoniae* Isolates?▽

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Using time-kill methodology, we investigated the interactions of an imipenem-colistin combination against 42 genetically distinct *Klebsiella pneumoniae* clinical isolates carrying a bla_{VIM-1}-type gene. Irrespective of the imipenem MIC, the combination was synergistic (50%) or indifferent (50%) against colistin-susceptible strains, while it was antagonistic (55.6%) and rarely synergistic (11%) against non-colistin-susceptible strains (with synergy being observed only against strains with colistin MICs of 3 to 4 μg/ml). The combination showed improved bactericidal activity against isolates susceptible either to both agents or to colistin.

During the past decade, VIM metallo-β-lactamases (MBLs) have spread rapidly among *Enterobacteriaceae* (4). MBL producers commonly exhibit a multiple-drug resistance phenotype as a result of combined chromosomally encoded or plasmid-mediated resistance mechanisms. Frequently, colistin and tigecycline remain the only therapeutic choices. Tigecycline has demonstrated in vitro activity against MBL producers (18), but evidence of in vivo efficacy against a variety of clinical infections (i.e., bacteremia or pneumonia) is still limited. On the other hand, randomized controlled trials supporting the use of colistin as a single-drug regimen, as well as studies on its pharmacokinetic/pharmacodynamic properties, are lacking (11). Recently, the emergence of colistin resistance among *Klebsiella pneumoniae* isolates further jeopardized the already limited treatment options in the intensive care unit setting (2). For all these reasons, combination therapies are frequently used in clinical practice, especially in hospitals with high rates of infections by MBL producers.

(Some of these data were presented at the 45th Infectious Disease Society Annual Meeting, 2007 [15a].)

We investigated the in vitro activities of imipenem and colistin alone and in combination against 42 unique clinical isolates of MBL-producing *K. pneumoniae* isolated in Greek hospitals from February 2004 to September 2006. MICs were determined by Etest (AB Biodisc, Solna, Sweden) and interpreted according to CLSI breakpoints for imipenem (3) and EUCAST breakpoints for colistin (7). The presence of a bla_{VIM} gene was confirmed by PCR (15). On the basis of PCR-restriction fragment length polymorphism analysis (9), all isolates carried a bla_{VIM-1}-type gene. Extended-spectrum β-lactamase production was detected with a modified CLSI confirmatory test (8). Genetic relatedness among studied isolates was evaluated with repetitive extragenic palindromic PCR methods (10). Patterns that differed by more than one amplification band were characterized as different. In vitro interactions between imipenem and colistin were tested using time-kill methodology. Antibiotic concentrations used were 10 μg/ml for imipenem (Merck, Rahway, NJ) and 5 μg/ml for colistin sulfate (Sigma, St. Louis, MO) because these concentrations represent the steady state achievable in human serum during treatment (12, 16) and thus are clinically relevant. For susceptible strains, if 4× MIC was not higher than 10 or 5 μg/ml for imipenem or colistin, respectively, this concentration was also tested.

Synergy was defined as a $\geq$2-$\log_{10}$ decrease in CFU/ml between the combination and the most active single agent at the different time points, with the number of surviving organisms in the presence of the combination being $\geq$2 $\log_{10}$ CFU/ml below the number of organisms in the starting inoculum. Antagonism was defined as a $\geq$2-$\log_{10}$ increase in CFU/ml between the combination and the most active single agent. All other interactions were characterized as indifferent. Bactericidal activity of single antibiotics or combinations was defined as a $\geq$3-$\log_{10}$ reduction in the CFU/ml of the initial inoculum after 24 h of incubation (1, 6). The lower limit of detection was 1.6 $\log_{10}$ CFU/ml. For analysis of the results, isolates were classified into four groups according to susceptibility to imipenem and colistin. The chi-square test was used to compare proportions of killing activity or synergy between groups by using Yates continuity correction in two-by-two tables. *P* values of $<$0.05 were considered statistically significant.

The results are shown in Table 1. The imipenem-colistin combination exhibited synergy against 12 of 24 (50%) colistin-susceptible MBL-producing *K. pneumoniae* isolates tested, but it was antagonistic against 10 of 18 (55.6%) non-colistin-susceptible isolates. Of note, isolates showing colistin MICs of 3 to 4 μg/ml behaved more like colistin-susceptible isolates, since in two of them (50%) a synergistic interaction was noted after exposure to the combination.

The combination was rapidly bactericidal against all isolates susceptible to both agents (n = 8) compared to imipenem and colistin alone (4× MIC), which were bactericidal against two and three isolates, respectively ($P < 0.05$). In the subgroup of 16 isolates that were non-imipenem-susceptible and colistin

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TABLE 1. MICs (μg/ml) of imipenem and colistin against bla_{VIM-1}-type MBL-producing *K. pneumoniae* isolates and in vitro interaction of the combination

Strain	MIC (μg/ml)		Presence of ESBL	Interaction[a] (time of growth [h])	No. of isolates showing synergy (or antagonism, if indicated)/total no. of isolates (%)
	Imipenem	Colistin			
Imipenem- and colistin-susceptible isolates					3/8 (37.5)
716	3	0.25	Yes	Indifference	
631 CI	0.75	0.38	Yes	Synergy (24)[b]	
2596 II	2	0.5	Yes	Synergy (24)[b]	
1057 B II	1.5	0.5	Yes	Indifference	
757	1	0.25	Yes	Synergy (3, 5, 24)[b]	
270 E II	1.5	0.25	Yes	Indifference	
2354	2	0.25	Yes	Indifference	
1037 E II	2	0.38	Yes	Indifference	
Non-imipenem-susceptible and colistin-susceptible isolates					9/16 (56.3)
350 I	6	0.5	No	Synergy (1, 24)[c]	
498 II	8	0.3	Yes	Synergy (24)[c]	
1587 I	>32	0.5	Yes	Synergy (24)[c]	
266 E	>32	0.4	Yes	Indifference	
1526	24	0.4	No	Synergy (24)[c]	
760 C	>32	0.4	No	Synergy (24)[c]	
329 B I	>32	0.2	Yes	Synergy (24)[c]	
175 III	>32	0.3	Yes	Indifference	
4412 B II	>32	0.5	Yes	Indifference	
377 II	>32	0.5	Yes	Synergy (5[c] or 5 and 24[d])	
513 E I	>32	0.38	Yes	Indifference	
682 E I	>32	0.19	Yes	Synergy (24)[c,d]	
735 E II	>32	0.38	Yes	Indifference	
1437 B II	>32	0.5	Yes	Synergy (24)[c]	
761 E I	>32	0.2	Yes	Indifference	
332 E	>32	0.25	Yes	Indifference	
Non-imipenem-susceptible and non-colistin-susceptible isolates					7/15 (46.7) (antagonism), 2/15 (13.3) (synergy)
748 A IV	>32	48	Yes	Indifference	
231 D	>32	48	Yes	Antagonism (3, 5)[d]	
1171 C II	12	256	Yes	Indifference	
1014 A I	>32	96	Yes	Antagonism (5)[d]	
1459	>32	256	Yes	Antagonism (3, 5)[d]	
1057 A	>32	48	Yes	Antagonism (5)[d]	
1326 A	>32	96	Yes	Antagonism (3, 5)[d]	
712 B I	24	16	No	Indifference	
1478 C I	8	128	Yes	Antagonism (3, 5)[d]	
4090 B	12	32	Yes	Indifference	
1110 B II	6	64	Yes	Antagonism (24)[d]	
674 C II	>32	3	No	Synergy (3, 5, 24)[d]	
963 II	>32	4	No	Indifference	
1919	>32	4	Yes	Synergy (24)[d]	
680 A	>32	4	No	Indifference	
Imipenem-susceptible and non-colistin-susceptible isolates					3/3 (100) (antagonism)
1119	2	48	Yes	Antagonism (3,[e] 5[d])	
318 G I	3	24	Yes	Antagonism (24)[d]	
240 B I	4	64	Yes	Antagonism (24)[d]	

[a] All combinations tested at all time points exhibited indifference unless otherwise specified.
[b] Concentrations tested were as follows: imipenem and colistin, 4× MIC.
[c] Concentrations tested were as follows: imipenem, 10 μg/ml; and colistin, 4× MIC.
[d] Concentrations tested were as follows: imipenem, 10 μg/ml; and colistin, 5 μg/ml.
[e] Concentrations tested were as follows: imipenem, 4× MIC; and colistin, 5μg/ml.

susceptible, the combination of imipenem (10 μg/ml) and colistin (4× MIC) was bactericidal against 10 (62.5%) isolates, while another combination of imipenem (10 μg/ml) and colistin (5 μg/ml) was bactericidal against 12 (75%) isolates compared to imipenem (10 μg/ml) and colistin (4× MIC) alone, which were bactericidal against zero and two isolates, respectively ($P < 0.05$). The antibiotic combination was bactericidal against only 2 of 15 (13.3%) isolates that were nonsusceptible to both imipenem and colistin. In the subgroup of isolates that were susceptible to imipenem but nonsusceptible to colistin

($n = 3$), the combination exhibited an antagonistic effect, and regrowth was noted for all isolates after 24 h of incubation. Overall, in the group of imipenem-susceptible isolates, imipenem alone at a concentration of 10 μg/ml or 4× MIC demonstrated killing activity against 7/11 (63.6%) or 2/8 (25%) isolates, respectively, at 24 h.

In order to evaluate the development of resistance as a reason for bacterial regrowth after 24 h of incubation with the studied combination, viable colonies were subjected to susceptibility testing in comparison with the respective wild-type strain, using agar dilution as described by CLSI (3). This evaluation was performed only for isolates that were initially susceptible to at least one of the tested antimicrobials.

For 7 of 12 isolates (58.3%) that were initially susceptible to colistin, a colistin-resistant clone (MIC range, 64 to >256 μg/ml) was selected after incubation with the tested combination. Conversely, among four isolates initially susceptible to imipenem that showed regrowth after 24 h of incubation with the combination, none developed resistance (MIC range, 1 to 4 μg/ml).

To our knowledge, the present study is the first to assess the in vitro interaction of imipenem and colistin against a large number of VIM-1-type MBL-producing *K. pneumoniae* isolates exhibiting a wide range of susceptibilities to these agents. Carbapenem resistance levels of MBL-positive *Enterobacteriaceae* are variable and often below the proposed resistance breakpoint (20) as a result of differences in outer membrane permeability or in the levels of VIM-1 production (13), but most experts recommend against the use of carbapenem monotherapy for treatment (4, 17), based on evidence of a strong inoculum effect in vitro (14). Other experimental data suggested that an increased imipenem dosage could be efficacious against susceptible isolates (5). In the era of multidrug resistance, our findings concerning the killing activity of imipenem as a single agent against selected susceptible MBL-producing strains merit further investigation. Importantly, the imipenem-colistin combination demonstrated improved bactericidal activity compared to either agent alone and yielded synergy against 14 of 42 (33.3%) *K. pneumoniae* isolates tested. Synergy was observed only against isolates exhibiting susceptibility or low-level resistance to colistin. In contrast, antagonism was observed against 10 of 42 (23.8%) strains tested, all of which exhibited high-level resistance to colistin. These differences underscore the importance of accurate susceptibility testing of colistin, with MIC determination. In concordance with these findings, the previous experiences of our group suggest that colistin-containing regimens are successful for the treatment of infections by VIM-1-type MBL-producing *Enterobacteriaceae* (19). The results of the present study merit further investigation in animal models and clinical trials. While waiting for these data, the coadministration of imipenem and colistin should probably be avoided for colistin-resistant VIM-producing *K. pneumoniae* because it could result in antagonism.

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