ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2010, p. 2732–2734 Vol. 54, No. 6
0066-4804/10/$12.00 doi:10.1128/AAC.01768-09


# *In Vitro* Double and Triple Bactericidal Activities of Doripenem, Polymyxin B, and Rifampin against Multidrug-Resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*[▽]

Carl Urban,[1,2]* Noriel Mariano,[1] and James J. Rahal[1,3]

*Infectious Disease Section, New York Hospital Queens, Flushing, New York,[1] and Departments of Microbiology[2] and Medicine,[3] Weill Cornell Medical College, New York, New York*

Received 14 December 2009/Returned for modification 12 February 2010/Accepted 28 March 2010

***In vitro* double and triple bactericidal activities of doripenem, polymyxin B, and rifampin were assessed against 20 carbapenem-resistant clinical isolates with different mechanisms of carbapenem resistance. Bactericidal activity was achieved in 90% of all bacteria assayed using combinations of polymyxin B, doripenem, and rifampin against five each of the carbapenem-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli* isolates studied. Combinations with these antibacterials may provide a strategy for treatment of patients infected with such organisms.**

Carbapenem resistance in *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* is acknowledged worldwide (1, 2, 4, 13, 15, 16). Mechanisms of carbapenem resistance in these bacteria can be due to a variety of carbapenemases alone and/or β-lactamases with porin protein mutations as well as other contributory strategies (13, 15). The latest carbapenem approved in the United States, doripenem, has demonstrated *in vitro* activity against a variety of multidrug-resistant (MDR) Gram-negative organisms, which produce well-characterized β-lactamases, and delayed development of resistance to doripenem has been demonstrated (6, 7, 8, 12). Combination therapy with several classes of antibiotics against multidrug-resistant pathogens has revealed increased activity over single agents and delayed development of resistance (14). This investigation studied the *in vitro* bactericidal activities of double and triple antibiotic combinations using doripenem (D), polymyxin B (PB), and rifampin (R) against *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* because of their progressive resistance to all available agents.

Twenty carbapenem-resistant clinical isolates with different mechanisms of carbapenem resistance and nonrelated by pulsed-field gel electrophoresis were studied, including five imipenem-resistant *K. pneumoniae* isolates (two with KPC and three with ACT-1 [AMPC-type] β-lactamases), five *A. baumannii* isolates (non-MBL or KPC β-lactamases), five *P. aeruginosa* isolates (one KPC and four non-MBL or KPC β-lactamases), and five *E. coli* isolates (one KPC-3 and four KPC-2 β-lactamases). Susceptibility of the isolates was initially determined by our clinical microbiology laboratory using the Phoenix system, and the results were confirmed in the infectious disease research laboratory using Etest methodology according to the manufacturer's specifications (bioMérieux North America). *E. coli* ATCC 25922 was tested as the control strain.

Bactericidal experiments were performed using double and triple antibiotic combinations of polymyxin B plus doripenem, polymyxin B plus rifampin, doripenem plus rifampin, and polymyxin B plus doripenem and rifampin as previously described (17). Time-kill studies were performed at concentrations at 1/4 of their MICs. Doripenem, polymyxin B, and rifampin alone were also tested at 1/4 MIC against each isolate. For bactericidal assays, samples were taken at time zero and 2, 4, 8, and 24 h. Aliquots were serially diluted, and a 10-μl aliquot was transferred onto plates, spread with a loop to minimize carryover to quantify bacterial counts, and incubated at 35°C for 24 h. Bactericidal activity was defined as a ≥3-log CFU/ml decrease in 24 h.

Genotypic and phenotypic characteristics for the carbapenem-resistant isolates used in this study are displayed in Table 1. All 20 isolates had the following MICs (μg/ml): for rifampin, ranging from 8 to >32; for ertapenem, >32; for doripenem, 1.5 to >32; for imipenem; 6 to >32; for meropenem, 2 to >32; and for polymyxin B, 0.5 to 12. The results of *in vitro* bactericidal activities and quantitative fold changes with double and triple antibiotic combinations are shown in Table 2. Combinations of polymyxin B-doripenem-rifampin at 1/4 MICs for each antibiotic were bactericidal for 4/5 *K. pneumoniae*, 3/5 *A. baumannii*, 5/5 *P. aeruginosa*, and 5/5 *E. coli* isolates. Combinations of polymyxin B-doripenem at 1/4 MICs for each antibiotic were bactericidal for 1/5 *K. pneumoniae*, 1/5 *A. baumannii*, 1/5 *P. aeruginosa*, and 4/5 *E. coli* isolates. Combinations of polymyxin B-rifampin at 1/4 MICs for each antibiotic were bactericidal for 1/5 *K. pneumoniae*, 2/5 *A. baumannii*, 1/5 *P. aeruginosa*, and 2/5 *E. coli* isolates. Combinations of doripenem-rifampin at 1/4 MICs for each antibiotic were bactericidal for 2/5 *K. pneumoniae*, 2/5 *A. baumannii*, 1/5 *P. aeruginosa*, and 1/5 *E. coli* isolates. Bactericidal activity was achieved in 85% of all bacteria assayed using combinations of polymyxin B-doripenem-rifampin, 30% with polymyxin B-doripenem, 30%

* Corresponding author. Mailing address: New York Hospital Queens, 56-45 Main Street, Flushing, New York 11355. Phone: (718) 670-1525. Fax: (718) 661-7899. E-mail: cmurban@nyp.org.
▽ Published ahead of print on 5 April 2010.

TABLE 1. Genotypic and phenotypic characteristics for carbapenem-resistant isolates[a]

| Etest isolate | Modified Hodge test | MBL Etest | Carbapenem resistance mechanism(s) | MIC (μg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | PB | RI | IP | MP | ERT | D |
| *K. pneumoniae* 1 | − | − | Porin + ACT-1 | 2 | >32 | >32 | 16 | >32 | 8 |
| *K. pneumoniae* 2 | − | − | Porin + ACT-1 | 1 | >32 | >32 | 32 | >32 | 32 |
| *K. pneumoniae* 3 | − | − | Porin + ACT-1 | 0.75 | >32 | >32 | 6 | >32 | 6 |
| *K. pneumoniae* 4 | + | − | KPC-2 | 1 | >32 | >32 | >32 | >32 | >32 |
| *K. pneumoniae* 5 | + | − | KPC-2 | 0.75 | >32 | 32 | 16 | >32 | 24 |
| *A. baumannii* 6 | − | − | ND | 1 | 8 | >32 | >32 | >32 | >32 |
| *A. baumannii* 7 | − | − | ND | 0.75 | >32 | >32 | >32 | >32 | >32 |
| *A. baumannii* 8 | − | − | ND | 1.5 | 16 | >32 | >32 | >32 | >32 |
| *A. baumannii* 9 | − | − | ND | 0.5 | 16 | >32 | >32 | >32 | >32 |
| *A. baumannii* 10 | − | − | ND | 1 | >32 | >32 | >32 | >32 | >32 |
| *P. aeruginosa* 11 | + | − | KPC + PCR | 2 | >32 | >32 | >32 | >32 | >32 |
| *P. aeruginosa* 12 | − | − | ND | 12 | >32 | >32 | >32 | >32 | 8 |
| *P. aeruginosa* 13 | − | − | ND | 1.5 | >32 | >32 | >32 | >32 | >32 |
| *P. aeruginosa* 14 | − | − | ND | 3 | >32 | >32 | >32 | >32 | 32 |
| *P. aeruginosa* 15 | − | − | ND | 2 | >32 | >32 | >32 | >32 | >32 |
| *E. coli* 16 | + | − | KPC-3 | 0.5 | >32 | >32 | 16 | >32 | 4 |
| *E. coli* 17 | + | − | KPC-2 | 1 | >32 | 6 | 3 | >32 | 2 |
| *E. coli* 18 | + | − | KPC-2 | 0.5 | >32 | 8 | 2 | 8 | 1.5 |
| *E. coli* 19 | + | − | KPC-2 | 1 | >32 | 6 | 2 | >32 | 1.5 |
| *E. coli* 20 | + | − | KPC-2 | 0.75 | >32 | 6 | 4 | 16 | 1.5 |

[a] PB, polymyxin B; RI, rifampin; IP, imipenem; MP, meropenem; ERT, ertapenem; D, doripenem; ND, not determined; +, positive result; −, negative result.

with doripenem-rifampin, and 25% with polymyxin B-rifampin at 1/4 MICs. Doripenem, polymyxin B, and rifampin tested alone, at 1/4 MIC, were not bactericidal.

Extreme drug resistance (XDR) and pan resistance in Gram-negative bacteria is being reported with increasing frequency (5, 10). Although combination therapy has been widely accepted for management of patients infected with *Mycobacterium tuberculosis* and human immunodeficiency virus, it has not been widely accepted for infections caused by multidrug resistant Gram-negative bacteria. Studies performed *in vitro* or in animal models often demonstrate synergy with antibiotic combinations, but few translate this success to the clinical arena because of the lack of well-controlled studies (3, 9, 11).

Our study showed that combinations of polymyxin B-doripenem-rifampin achieved 100% bactericidal activity, defined as a ≥3-log-CFU/ml decrease in 24 h at 1/4 MICs for *P. aeruginosa* and *E. coli*, 80% for *K. pneumoniae*, and 60% for *A. baumannii* despite resistance to the carbapenems and rifampin alone. A previous study using similar methodology demonstrated that a combination of polymyxin B at 0.5 times the MIC plus rifampin had synergistic activity against 15/16 KPC-producing *Klebsiella pneumoniae* isolates and synergistic bacteri-

TABLE 2. Logarithmic and fold changes of time-kill experiments at 24 h in various drug combinations with 1/4 MIC[a]

| Isolate | PB-D-RI | | PB-D | | PB-RI | | D-RI | |
|---|---|---|---|---|---|---|---|---|
| | LogΔ (CFU/ml) | Fold change | LogΔ (CFU/ml) | Fold change | LogΔ (CFU/ml) | Fold change | LogΔ (CFU/ml) | Fold change |
| *K. pneumoniae* 1 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 |
| *K. pneumoniae* 2 | −2.70 | 500 | −2.10 | 125 | +3.54 | 35,000 | ≤−3.70[b] | ≥5,000 |
| *K. pneumoniae* 3 | ≤−3.70[b] | ≥5,000 | −0.84 | 7 | −0.65 | 4.4 | +1.51 | 32 |
| *K. pneumoniae* 4 | ≤−3.70[b] | ≥5,000 | +3.79 | 6,100 | +4.30 | 20,000 | +4.30 | 20,000 |
| *K. pneumoniae* 5 | ≤−3.70[b] | ≥5,000 | −2.70 | 500 | +3.45 | 2,793 | +3.07 | 1,188 |
| *A. baumannii* 6 | ≤−3.70[b] | ≥5,000 | +0.19 | 1.5 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 |
| *A. baumannii* 7 | −2.0 | 100 | +5.3 | 200,000 | +0.87 | 7.4 | +5.30 | 200,000 |
| *A. baumannii* 8 | −1.67 | 46 | +0.56 | 3.7 | +2.43 | 267 | ≤−3.70[b] | ≥5,000 |
| *A. baumannii* 9 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | +0.8 | 6.3 |
| *A. baumannii* 10 | ≤−3.70[b] | ≥5,000 | +5.05 | 112,000 | +5.30 | 200,000 | +4.0 | 10,000 |
| *P. aeruginosa* 11 | ≤−3.70[b] | ≥5,000 | +1.56 | 36.5 | +2.95 | 886 | +0.52 | 3.3 |
| *P. aeruginosa* 12 | ≤−3.70[b] | ≥5,000 | +4.60 | 39,900 | ≤−3.70[b] | ≥5,000 | +4.17 | 14,700 |
| *P. aeruginosa* 13 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | +4.30 | 20,000 | ≤−3.70[b] | ≥5,000 |
| *P. aeruginosa* 14 | ≤−3.70[b] | ≥5,000 | +5.30 | 200,000 | +4.30 | 20,000 | +2.66 | 460 |
| *P. aeruginosa* 15 | ≤−3.70[b] | ≥5,000 | +4.75 | 56,000 | +4.30 | 20,000 | +3.93 | 8,600 |
| *E. coli* 16 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | −1.81 | 63.8 | +4.32 | 21,100 |
| *E. coli* 17 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | +5.30 | 200,000 |
| *E. coli* 18 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | +2.21 | 161 | +5.03 | 106,000 |
| *E. coli* 19 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 | ≤−3.70[b] | ≥5,000 |
| *E. coli* 20 | ≤−3.70[b] | ≥5,000 | +0.37 | 2.4 | +1.72 | 53 | +3.56 | 3,633 |

[a] PB, polymyxin B; D, doripenem; RI, rifampin.
[b] Maximum log reduction in CFU/ml detectable by assay = (standard inoculum) 5.7 $\log_{10}$ − (lower limit of detection) 2.0 $\log_{10}$.

cidal activity against 10/16 of the isolates using a combination of polymyxin B plus imipenem (2). While *in vitro* studies do not always correlate with *in vivo* efficacy, our study showed that bactericidal activity was achieved in 85% of our multidrug resistant isolates at 1/4 their MICs. Administration of approved doses of each of the antibiotics would be in excess of the concentrations used in this *in vitro* study. In an era of burgeoning multidrug resistance, including that against carbapenems, triple combinations with these antibacterials may provide a strategy for treatment of patients infected with such organisms.

This study was funded by a grant from Johnson & Johnson Pharmaceutical Research and Development and by BMA Medical Foundation, Inc.

## REFERENCES


1. **Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale.** 2004. Emergence of carbapenem-resistant *Klebsiella* spp. possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-lactamases in New York City. Clin. Infect. Dis. **39:**55–60.
2. **Bratu, S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D. Landman.** 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and *in vitro* activity of polymyxin B and other agents. J. Antimicrob. Agents **56:**128–132.
3. **Chow, J. W., and V. L. Yu.** 1999. Combination antibiotic therapy versus monotherapy for gram-negative bacteremia: a commentary. Int. J. Antimicrob. Agents **11:**7–12.
4. **Deshpande, L. M., P. R. Rhomberg, H. S. Sader, and R. N. Jones.** 2006. Emergence of serine carbapenemases (KPC and SME) among clinical strains of *Enterobacteriaceae* isolated in the United States medical centers: report from the MYSTIC program (1999–2005). Diagn. Microbiol. Infect. Dis. **56:**367–372.
5. **Elemam, A., J. Rahimian, and W. Mandell.** 2009. Infection with panresistant *Klebsiella pneumoniae*: a report of 2 cases and a brief review of the literature. Clin. Infect. Dis. **49:**271–274.
6. **Huynh, H. K., D. J. Biedenbach, and R. N. Jones.** 2006. Delayed resistance selection for doripenem when passaging *Pseudomonas aeruginosa* isolates with doripenem plus an aminoglycoside. Diagn. Microbiol. Infect. Dis. **55:** 241–243.
7. **Jones, R. N., H. S. Sader, and T. R. Fritsche.** 2005. Comparative activity of doripenem and three other carbapenems tested against gram-negative bacilli with various β-lactamase resistance mechanisms. Diagn. Microbiol. Infect. Dis. **52:**71–74.
8. **Mushtaq, S., Y. Ge, and D. M. Livermore.** 2004. Comparative activities of doripenem versus isolates, mutants, and transconjugants of *Enterobacteriaceae* and *Acinetobacter* spp. with characterized β-lactamases. Antimicrob. Agents Chemother. **48:**1313–1319.
9. **Ostenson, R. C., B. T. Fields, and C. M. Nolan.** 1977. Polymyxin B and rifampin: new regimen for multiresistant *Serratia marcescens* infections. Antimicrob. Agents Chemother. **12:**655–659.
10. **Paterson, D. L., and Y. Doi.** 2007. A step closer to extreme drug resistance (XDR) in gram-negative bacilli. Clin. Infect. Dis. **45:**1179–1181.
11. **Petrosillo, N., E. Ioannidou, and M. E. Falagas.** 2008. Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies. Clin. Microbiol. Infect. **14:**816–827.
12. **Queenan, A. M., W. Shang, R. Flamm, and K. Bush.** 2010. Hydrolysis and inhibition profiles of β-lactamases from molecular classes A to D with doripenem, imipenem and meropenem. Antimicrob. Agents Chemother. **54:**565–569.
13. **Queenan, A. M., and K. Bush.** 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. **20:**440–458.
14. **Rahal, J. J.** 2006. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. Clin. Infect. Dis. **43**(Suppl. 2)**:**S95–S99.
15. **Urban, C., and J. J. Rahal.** 2004. Mechanisms of carbapenem resistance in *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Rev. Med. Microbiol. **15:**63–72.
16. **Urban, C., P. A. Bradford, M. Tuchman, S. Segal-Maurer, W. Wehbeh, L. Grenner, R. Colon-Urban, N. Mariano, and J. J. Rahal.** 2008. Carbapenem-resistant *Escherichia coli* harboring *Klebsiella pneumoniae* carbapenemase β-lactamases associated with long-term care facilities. Clin. Infect. Dis. **46:** e127–e130.
17. **Yoon, J., C. Urban, C. Terzian, N. Mariano, and J. J. Rahal.** 2004. *In vitro* double and triple synergistic activity of polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **48:**753–758.