1574

Delayed Resistance Selection for Doripenem (DOR) when Passaging *P. aeruginosa* (PSA) Isolates With an Aminoglycoside

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AMENDED ABSTRACT

Background:
DOR (MIC_{50}, 0.5 mg/L), a parenteral carbapenem, has activity equal or superior to meropenem (MIC_{50}, 0.5) and imipenem (MIC_{90}, 1) against PSA. This study determined the resistance selection of DOR ± gentamicin (GENT) during subinhibitory passaging using 6 PSA strains.

Methods:
PSA bloodstream strains (2003) with DOR MICs at or near the proposed susceptible breakpoint (≤ 4 mg/L) were selected for testing. Broth microdilution methods were used to establish baseline MICs for DOR (2-8) and GENT (4->256). After overnight incubation, the organism suspension, one well below the MIC was transferred into fresh media and allowed to grow (0.5 McFarland) then inoculated into panels containing DOR±GENT (MIC/4; not to exceed 4 mg/L). Isolates were passaged over 7 days.

Results:
The table shows baseline doripenem MIC values and 7 day passaging results:

		DOR/+GENT MIC (mg/L) by passage						
Strain #	Baseline	Day 1	2	3	4	5	6	7
30-3232A	2	2/2	2/1	2/2	4/4	2/4	4/4	2/8
107-3347A	4	4/2	4/2	16/4	>16/4	16/4	>16/4	>16/8
45-11372A	4	4/2	16/4	16/4	>16/8	>16/4	>16/8	>16/8
75-3075A	4	8/2	8/2	8/2	8/4	8/4	8/4	16/4
24-3338A	8	8/2	8/2	8/4	16/8	16/8	16/8	16/8
38-12060A	8	8/8	8/8	16/8	16/8	8/8	8/8	8/8

After 7 days, strain MIC increases were: DOR alone at ≥ 4X (3), 2X (1), NC (2); + GENT at 4X (1), 2X (2), NC (3). No strain was resistant to DOR+GENT (MIC, ≥ 16 mg/L) and enhanced combination activity was noted for nearly all strains during the first 3 passages.

Conclusions:
The combination of a co-drug (GENT) with DOR against PSA provided enhanced activity during early passaging and minimized the number of DOR-R strains. DOR and aminoglycoside combinations may be an effective treatment regimen for infections caused by PSA with elevated carbapenem MIC values.

INTRODUCTION

The treatment of infections caused by *P. aeruginosa* can be difficult due to pre-existing or emergent multidrug-resistance patterns among some strains. Combination therapy is often suggested when treating this pathogen and many studies have documented in vitro synergistic and additive effects when various antimicrobial agents are tested in combination. It has been shown that ß-lactams used in combination with aminoglycosides can be synergistic against Gram-negative organisms, including *P. aeruginosa,* and patients have improved clinically when given these combinations. Carbapenems are commonly prescribed to patients with *P. aeruginosa* infections given the high rates of resistance often found to other antimicrobial classes. However, rapid development of resistance to carbapenems even using combination therapies has been documented and some studies suggest antipseudomonal penicillins may inactivate aminoglycosides.

Doripenem is a new carbapenem that shows equal or superior activity to other drugs in this class, including imipenem and meropenem against *P. aeruginosa*. In this study, strains of *P. aeruginosa* were passaged over the course of one week in doripenem alone and in the presence of gentamicin. This was done to determine the effect on the doripenem MIC values after repeated exposure to the drug and whether an aminoglycoside could maintain the baseline doripenem MIC during this passaging experiment. In addition, the antimicrobial activity of several drugs, including doripenem, against recent *P. aeruginosa* strains from three geographic regions were evaluated.

MATERIALS AND METHODS

Bacterial isolates. Strains of *P. aeruginosa* were collected and tested during the 2003 SENTRY Antimicrobial Surveillance Program from patients with documented infections of the bloodstream, respiratory tract, skin and soft tissues, and the urinary tract among others. A total of 834 strains were referred for reference MIC testing from Europe (453), North America (228) and Latin America (153). Six strains collected from patients with bloodstream infections were selected for the passaging experiment that had MIC values to doripenem that were near the proposed breakpoint for susceptibility (≤ 4 mg/L; similar to that of other carbapenem antibiotics). The MIC values to gentamicin for most of these isolates were also selected near the susceptibility breakpoint (≤ 4 mg/L). One isolate was highly resistant to the aminoglycosides tested (gentamicin, tobramycin and amikacin).

Susceptibility test methods. The susceptibility profiles of the strains were determined using broth microdilution testing according to CLSI (formerly NCCLS) methods. Tests were performed in Mueller-Hinton broth using a 0.5 McFarland Standard suspension of organism inoculum to determine the baseline MIC values. Following overnight incubation, the MIC results were determined visually and the well below the MIC was extracted and transferred to fresh Mueller-Hinton broth and grown to a 0.5 McFarland density which was used as the test inoculum for the next passage in antibiotic-containing media. Strains were passaged over seven days in doripenem and in the combination of doripenem and gentamicin at one fourth the MIC value of gentamicin.

Table 1. Activity of doripenem and eight comparator agents tested against *P. aeruginosa* isolated in Europe, North America and Latin America in 2003.

	MIC (mg/L)				
Region (no. strains)/antimicrobial agent	50%	90%	Range	% susceptible[a]	% resistant[a]
Europe (453)					
Doripenem	0.5	8	0.03->16	85.2	8.6
Imipenem	1	>8	≤0.5->8	79.9	15.0
Meropenem	0.5	16	0.016->16	82.3	12.6
Ceftazidime	4	>16	≤1->16	73.3	21.2
Cefepime	4	>16	≤0.12->16	74.6	13.7
Piperacillin/Tazobactam	8	256	0.5->256	79.5	20.5
Aztreonam	8	>16	0.25->16	63.4	22.5
Gentamicin	≤2	>8	≤2->8	69.5	28.5
Ciprofloxacin	0.25	>4	≤0.03->4	69.1	28.5
North America (228)					
Doripenem	0.5	2	0.06->16	96.1	2.2
Imipenem	1	4	≤0.5->8	90.8	5.7
Meropenem	0.5	4	0.06->16	93.0	3.9
Ceftazidime	2	16	≤1->16	87.3	6.6
Cefepime	2	16	0.5->16	89.5	3.1
Piperacillin/Tazobactam	4	32	0.5->256	92.5	7.5
Aztreonam	8	>16	0.5->16	74.1	16.2
Gentamicin	≤2	4	≤2->8	90.4	8.8
Ciprofloxacin	0.12	>4	≤0.03->4	78.1	18.0
Latin America (153)					
Doripenem	1	8	0.03->16	76.5	9.8
Imipenem	1	>8	≤0.5->8	66.0	21.6
Meropenem	1	16	0.03->16	71.2	20.9
Ceftazidime	4	>16	≤1->16	60.8	33.3
Cefepime	8	>16	0.5->16	62.1	19.0
Piperacillin/Tazobactam	8	256	≤0.12->256	71.9	28.1
Aztreonam	8	>16	0.25->16	51.0	28.8
Gentamicin	≤2	>8	≤2->8	61.4	35.9
Ciprofloxacin	0.25	>4	0.06->4	62.1	37.3

a. Susceptibility percentages were based upon CLSI M100-S15 breakpoint criteria. Breakpoint criteria have not been established for doripenem and percentages are based upon expected breakpoints (≤ 4 susceptible, ≥ 16 resistant) similar to those of the other carbapenems.

RESULTS

- Doripenem (MIC_{90}, 2 - 8 mg/L) was two-fold more potent than imipenem and meropenem against *P. aeruginosa* strains isolated in Europe, North America and Latin America (Table 1).
- Considerable differences in the susceptibility patterns for *P. aeruginosa* isolates collected from the three regions were apparent. Doripenem was the most active compound tested with susceptibility rates of 85.2%, 96.1% and 76.5% in Europe, North America and Latin America, respectively. Imipenem (66.0 - 90.8% susceptible) and meropenem (71.2 - 93.0% susceptible) were less active (Table 1).
- The isolates that were used in the passage experiment had MIC values that were near the expected MIC breakpoint for doripenem (Table 2). All but one isolate was also near the CLSI breakpoints for the aminoglycosides.
- Following seven days of passaging in doripenem alone, four of the six isolates had doripenem MIC values that increased two- to ≥ eight-fold and two strains maintained the baseline doripenem MIC. In the presence of gentamicin, three isolates had original doripenem MIC values, two strains had a two-fold increase and one strain increased four-fold.

Table 2. ß-lactam and aminoglycoside MIC results for six *P. aeruginosa* isolates selected for passaging experiments.

	Antimicrobial agents (mg/L)							
Strain #	Ceftazidime	Cefepime	Doripenem	Imipenem	Meropenem	Gentamicin	Tobramycin	Amikacin
30-3232A	4	8	2	8	8	4	8	16
107-3347A	4	16	4	>8	16	4	1	8
45-11372A	4	16	4	8	16	8	2	16
75-3075A	4	16	4	8	16	8	1	16
24-3338A	>16	16	8	>8	8	4	1	8
38-12060A	>16	>16	8	>8	16	>256	>16	>32

Table 3. Seven day passage experiment with six *P. aeruginosa* isolates in the presence of doripenem alone and in combination with gentamicin.

	Doripenem/+ gentamicin MIC (mg/L) by passage								
	Baseline		Days						
Strain #	Doripenem	Gentamicin	1	2	3	4	5	6	7
30-3232A	2	4	2/2	2/1	2/2	4/4	2/4	4/4	2/8
107-3347A	4	4	4/2	4/2	16/4	>16/4	16/4	>16/4	>16/8
45-11372A	4	8	4/2	16/4	16/4	>16/8	>16/4	>16/8	>16/8
75-3075A	4	8	8/2	8/2	8/2	8/4	8/4	8/4	16/4
24-3338A	8	4	8/2	8/2	8/4	16/8	16/8	16/8	16/8
38-12060A	8	>256	8/8	8/8	16/8	16/8	8/8	8/8	8/8

CONCLUSIONS

- Resistance to commonly prescribed anti-pseudomonal agents is high in Latin America and Europe. The carbapenems were the most active agents tested in all regions (doripenem > meropenem > imipenem) against *P. aeruginosa*.
- The combination of gentamicin and doripenem provided an advantage based on the doripenem MIC values obtained after seven days of passaging, although modest increases (two- to four-fold) were detected.
- Carbapenem and aminoglycoside combination therapy may be appropriate to provide a delayed resistance selection in patients with *P. aeruginosa* infections.

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