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Original article

Effectiveness of antibiotic combination therapy as evaluated by the Break-point Checkerboard Plate method for multidrug-resistant *Pseudomonas aeruginosa* in clinical use

Itaru Nakamura [a], Tetsuo Yamaguchi [a,b,*], Ayaka Tsukimori [a], Akihiro Sato [a], Shinji Fukushima [a], Yasutaka Mizuno [a], Tetsuya Matsumoto [a,b]

[a] Department of Infection Control and Prevention, Tokyo Medical University Hospital, Japan
[b] Department of Microbiology, Tokyo Medical University, Japan

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ABSTRACT

Multidrug-resistant *Pseudomonas aeruginosa* (MDRP) strains are defined as having resistance to the following 3 groups of antibiotics: carbapenems, aminoglycosides, and fluoroquinolones. Antibiotic combinations have demonstrated increased activity *in vitro* compared with a single agent. As an *in vitro* method of determining the combination activity of antibiotics, the Break-point Checkerboard Plate (BC-plate) can be used routinely in clinical microbiology laboratories. We evaluated the effectiveness of the BC-plate for MDRP infections in clinical settings.

We retrospectively selected cases of MDRP infection treated with combination therapy of antibiotics in Tokyo Medical University Hospital (1015 beds), Tokyo, Japan, from November 2010 to October 2012.

A total of 28 MDRP strains were clinically isolated from 28 patients during the study period. This study design is a case series of MDRP infection. Six infections among the 28 patients were treated based on the results of the BC-plate assay, and the 6 strains tested positive for MBL. One patient had pneumonia, 3 had urinary tract infections, 1 had vertebral osteomyelitis, and 1 had nasal abscess. The combination of aztreonam with amikacin demonstrated the most frequently recognized *in vitro* effect (5 patients). Next, aztreonam with ciprofloxacin and piperacillin with amikacin revealed equivalent *in vitro* effects (3 patients, respectively). The clinical cure rate was 83.3% (5/6 patients).

Antibiotic combination therapy based on the results of the BC-plate assay might indicate the effective therapy against MDRP infection in clinical settings.

1. Introduction

Pseudomonas aeruginosa infections continue to pose a therapeutic dilemmma because of the wide range of acute and chronic infections, including wound and pulmonary infections, and even sepsis. Although the definition of multidrug-resistant *P. aeruginosa* (MDRP) is diverse in previous reports, it is commonly defined as having resistance to the following groups of antibiotics: carbapenems, aminoglycosides, and fluoroquinolones. It has emerged as an increasingly problematic cause of hospital-acquired infection. Several antibiotic combinations against MDRP strains have demonstrated increased activity *in vitro* compared with a single agent.

There are 2 laboratory methods commonly used to determine synergism. The first is the use of the fractional inhibitory concentration (FIC) index, which is determined by either the broth or agar checkerboard techniques [1]. The other method is the use of the time–kill curve to compare differences in colony counts of an organism over a determined time interval. However, the time-consuming and labor-intensive nature of these tests makes them unsuitable for routine use. As an *in vitro* method of determining the activity of combination antibiotics at clinically achievable levels, the Break-point Checkerboard Plate (BC-plate) was described [2]. This method uses designated combinations of the following 8 antibiotics: ceftazidime (CAZ), piperacillin (PIPC), meropenem (MEPM), aztreonam (AZT), gentamicin (GM), ciprofloxacin (CPFX), colistin, and rifampicin (RFP). Two concentrations of each antibiotic were selected by considering the breakpoints of these antibiotics, in

* Corresponding author. Department of Microbiology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan. Tel.: +81 3 3351 6141x241, fax: +81 3 3351 6160.
E-mail address: t_yama@tokyo-med.ac.jp (T. Yamaguchi).

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addition to tissue and serum concentrations. This method can screen multiple antibiotic combinations.

However, the effectiveness in clinical use of antibiotic combination therapy indicated by the BC-plate method for MDRP infections is unclear. To the best of our knowledge, treatments for only a few clinical cases of MDRP infection based on BC-plate results have been reported. In this study, we evaluated the effectiveness of antibiotic combination therapy for MDRP infections in clinical settings based on the BC-plate method.

2. Materials and methods

We retrospectively selected MDRP patients treated with combination therapy of antipseudomonal antibiotics at Tokyo Medical University Hospital (1015 beds), Tokyo, Japan, from November 2010 to October 2012. MDRP strains were defined as having resistance to the following 3 antibiotics: IPM or MEPM (MIC $\geq$ 16 μg/mL), AMK (MIC $\geq$ 32 μg/mL), and CPFX (MIC $\geq$ 4 μg/mL).

The MDRP strains were 28 isolated strains from the clinical specimens of 28 patients during this study period. Among the 28 patients, 6 acquired infections due to MDRP. Other patients had no infections or treatment. All 6 patients were treated with antipseudomonal combination therapy based on the results of the BC-plate assay. This study design is a case series of MDRP infection treated with combination therapy. These 6 patients were from 4 hospital wards, including 3 in hematology, 1 in gastroenterology, 1 in urology and 1 in gynecology. The patients from hematology were hospitalized at a different time.

Antimicrobial susceptibility and MICs were determined using the broth microdilution method. Antimicrobial agents included PIPC, CAZ, AZT, imipenem/cilastatin (IPM/CS), MEPM, amikacin (AMK), and CPFX. Strains were tested for the production of metallo-β-lactamase (MBL) using a commercial disk (Eiken Chemical Co., Tokyo, Japan) containing 3 mg of sodium mercaptoacetic acid [3].

The resistant genes in clinical isolates were detected by PCR using the following primers [4,5]: for *aac(6′)-Iae* they were aacS1-F (5′-cgcaagctgcagaaattctat-3′) and aacS1R (5′-tcccatttgcattaggaatca-3′); for *aac(6′)-Ib* they were aac_up (5′-tgaccttgcgatgctctatg-3′) and aac_dw (5′-ttaggcatcactgcgtgttc-3′); for bla_{IMP} they were IMP-F (5′-dttyctaaacayggyttggt-3′) and IMP-R (5′-ytttyaggyarccaaacyact-3′).

The combination activity was measured by the BC-plate method. It is difficult to define and assess the synergistic effect of the BC-plate method without using the FIC index or the time–kill curve. Therefore, in this study, combination activity was defined as inhibition in the culture well being observed with antibiotic combination, in comparison with the inhibition by a single agent. The diagnosis of the infection, underlying diseases, antibiotic combination actually used, duration of antibiotic combination therapy, adverse events, and outcome were evaluated retrospectively. Clinical cure was defined as the removal of symptoms and signs of infection.

3. Results

Table 1 shows the MICs (μg/mL) of the 8 drugs against the 6 strains of MDRP. All 6 strains produced MBL and were positive for bla_{IMP} and *aac(6′)-Ib*.

One patient had pneumonia, 3 had urinary tract infections, 1 had abdominal abscess, 1 had vertebral osteomyelitis, and 1 had nasal abscess. The actual antibiotic combinations to be used based on the results of the BC-plate assay were 2 AZT + AMK, 1 AZT + ABK, 1 AZT + colistin, 1 AZT + CPFX, and 1 PIPC/TAZ + AMK. The combination of AZT with AMK demonstrated the most frequently recognized *in vitro* effect in 5 patients. Next, AZT with CPFX and PIPC with AMK revealed equivalent *in vitro* effects in 3 patients, respectively. The clinical characteristics of these 6 patients with MDRP infections and the results of the BC-plate tests are shown in Table 2.

Table 1
MICs (mg/dL) of 8 antipseudomonal agents against 6 strains of MDRP.

	PIPC	CAZ	AZT	IPM/CS	MEPM	AMK	CPFX	Colistin
Patient 1	32	>64	16	64	64	64	32	<2
Patient 2	64	>64	16	64	128	32	32	<2
Patient 3	32	>64	32	>64	>128	>64	>32	<2
Patient 4	>128	>64	32	32	>128	>64	>32	2
Patient 5	>128	>64	32	32	64	32	4	<2
Patient 6	>128	>64	32	8	64	32	32	<2

All 6 strains were susceptible to colistin. Because no combination selection treatment was effective without colistin in patient 4, the combination therapy included colistin. In the other 5 patients, combination activity of agents without colistin was observed.

Regarding adverse events at the end of combination therapy, 3 patients had renal dysfunction (>0.3 mg/dL increase in serum creatinine level since the start of combination treatment). However, there were no patients with severe renal failure. Moreover, auditory impairment was not observed. The clinical cure rate was 83.3% (5/6 patients). The cause of death for patient 6 was not a worsening of the MDRP infection, but rather a progression of ovarian cancer.

4. Discussion

In Japan, where intravenous colistin cannot be used, combination antibiotic therapy is expected to be effective for the treatment of MDRP. However, the common methods for measuring antibiotic combination effects, the FIC index or time–kill curve, are time-consuming and labor-intensive. Choosing combination agents in a simple and timely manner is important in the clinical setting. In Japan, a commercially available “Break-point Checkerboard Plate” is the BC-plate Eiken. As far as our search of reports in English revealed, only 3 patients with MDRP infection treated with the combination therapy selected using the BC-plate have been reported in Japan [6]. We described 6 patients with MDRP infection treated with the combination antibiotic therapy based on the BC-plate assay and revealed good clinical responses. This information might provide clinical implications regarding the use of the BC-plate for MDRP infections.

In our study, AZT with AMK, AZT with CPFX or PIPC with AMK revealed relative combination activity. Although several antibiotics, including AZT and AMK, were actually selected as combination regimens, each infected patient was treated with a different drug combination depending on each clinical setting. This indicates the advantage of the BC-plate, allowing evaluation of the effect of combination regimens by 8 agents on a single plate at one time and enabling selection depending on each clinical situation.

Strains producing IMP-type MBL often remain susceptible to monobactams [7]. Araoka et al. have reported that AZT and aminoglycoside antibiotic combinations decreased the MICs of AZT in a dose-dependent manner [8]. Oie et al. have reported that the combination of AZT and AMK was the most effective, inhibiting proliferation in MDRP [9]. Therefore, the concomitant use of monobactams and aminoglycosides seems to be promising. As a result, combination regimens using monobactams and aminoglycosides were often selected in previous studies and in ours. These combinations seem to be effective clinically. AZT with aminoglycosides is considered a key drug combination.

In our study, *blaIMP* was positive, and the type of *AAC(6′)* was Ib. Although the favorable cure rate in our study might contribute to that, no previous reports indicated the results of *blaIMP* or *ACC(6′)*