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In vitro Effects of Various Antimicrobials Alone and in Combinations against Imipenem-Resistant *Pseudomonas aeruginosa*

(Kesan *In vitro* Pelbagai Antimikrob Sendiri dan Gabungan terhadap Rintangan-Imipenem *Pseudomonas aeruginosa*)

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ABSTRACT

Imipenem-resistant Pseudomonas aeruginosa *(IRPA) infection is a serious problem in hospitals. Combination therapy is an alternative treatment for this infection. In this study, the* in vitro *activities of amikacin, aztreonam, ceftazidime, ciprofloxacin, colistin, imipenem, and piperacillin/tazobactam alone and in various combinations were determined by E-test for 38 imipenem-resistant* P. aeruginosa *isolates obtained from a Thai hospital. Of the 38 IRPA isolates, 9 (24%) were low-level IRPA (defined as MICs of imipenem 8-32 µg/mL) and 29 (76%) were high-level IRPA (defined as MICs of imipenem >32 µg/mL). The high-level IRPA isolates were susceptible to colistin (90%), piperacillin/tazobactam (72%), and amikacin (52%). The low-level IRPA isolates were susceptible to colistin (100%) and all other antimicrobials tested (78%-89%). The MIC_{50} value of colistin against both the high-level and low-level IRPA isolates was 1.5 µg/mL. Of all the antimicrobial combinations tested, ceftazidime plus ciprofloxacin displayed the highest percentages of synergistic effects against IRPA isolates (26%, 10/38 isolates) and a high percentages of synergistic effects against high-level IRPA isolates (21, 6/29 isolates), with no antagonistic effects detected. Colistin had the greatest activity against most IRPA isolates among all of the antimicrobials tested, while ceftazidime plus ciprofloxacin showed promise in treating infections caused by IRPA isolates including high-level IRPAs.*

Keywords: Etest; imipenem resistant; Pseudomonas aeruginosa; *synergy*

ABSTRAK

Jangkitan rintangan-imipenem Pseudomonas aeruginosa *(IRPA) merupakan masalah yang serius di hospital. Terapi gabungan adalah rawatan alternatif bagi jangkitan ini. Dalam kajian ini, aktiviti* in vitro *amikasin, aztreonam, ceftazidime, ciprofloxacin, colistin, imipenem dan piperacillin/tazobactam semata-mata serta pelbagai gabungan ditentukan oleh ujian E-38 rintangan-imipenem pencilan* P. aeruginosa yang *diperoleh daripada sebuah hospital di Thailand. Dalam pencilan 38 IRPA, 9 (24%) ialah IRPA tahap rendah (ditakrifkan sebagai MICs imipenem 8-32 µg/mL) dan 29 (76%) IRPA tahap tinggi (ditakrifkan sebagai MICs imipenem > 32 µg/mL). Pencilan IRPA tahap tinggi telah menyebabkan ia terdedah kepada colistin (90%), piperacillin/tazobactam (72%) dan amikasin (52%). Pencilan IRPA tahap rendah rentan kepada colistin (100%) dan semua ujian antimikrob (78% - 89%). Nilai colistin MIC_{50} terhadap kedua-dua pencilan di peringkat tinggi dan IRPA tahap rendah adalah 1.5 µg/mL. Daripada semua kombinasi antimikrob yang diuji, ceftazidime dan ciprofloxacin menunjukkan peratusan tertinggi kesan bersinergisma terhadap pencilan IRPA (26%, pencilan 10/38) dan tinggi peratusan daripada kesan bersinergisma terhadap tahap tinggi IRPA terasing (21%, pencilan 6/29) dengan tiada kesan berantagonis dikesan. Colistin menunjukkan aktiviti terbesar berbanding kebanyakan pencilan IRPA antara semua antimikrob yang diuji, manakala ceftazidime dan ciprofloxacin menunjukkan keupayaan dalam merawat jangkitan yang disebabkan oleh pencilan IRPA termasuk IRPAs tahap tinggi.*

Kata kunci: Etest; Pseudomonas aeruginosa; *rintangan imipenem; sinergi*

INTRODUCTION

Pseudomonas aeruginosa is a nosocomial pathogen and cases involving such infections have become a serious problem in hospitals, especially in critically ill and immunocompromised patients. *P. aeruginosa* is frequently identified as the cause of surgical site infections (18%), respiratory tract infections (17%), and bloodstream infections (8%) (Lister et al. 2009; Tolera et al. 2018). This pathogen can survive in various environments such as in variable pH and temperature conditions. *P. aeruginosa* endosymbionts have also been detected in *Acanthamoeba* hosts from dust samples in air-conditioning vents in wards and operating theatres (Faizah et al. 2017). *P. aeruginosa* has been found to be resistant to several antipseudomonal agents (β-lactams, aminoglycosides, and fluoroquinolones). The mechanisms of resistance are associated with β-lactamases, decreased bacterial wall permeability, target alterations, and efflux pumps (Lister et al. 2009).

Imipenem is used in the empirical treatment of *Pseudomonas* infections. However, imipenem-resistant

strains have been detected during therapy (Kanj & Kanafani 2011). In Asian countries, a recent study found the resistance rate of *P. aeruginosa* to imipenem was 27.2% (Kang & Song 2013). Resistance to imipenem in *P. aeruginosa* can be due to decreased outer membrane permeability by loss of the OprD porin, increased efflux systems, or metallo- β-lactamases (Laupland et al. 2005; Lister et al. 2009).

Combination therapy has recently been suggested as an alternative for the treatment of severe *P. aeruginosa* infections as it has shown increased bactericidal activity, reduced toxicity, and lower rates of resistant strains during therapy (Moore & Flaws 2011; Song et al. 2003).

Previous *in vitro* studies have reported that combinations of antipseudomonal agents such as ceftazidime plus amikacin (Song et al. 2003), ceftazidime plus ciprofloxacin (Altoparlak et al. 2005), tazobactam/piperacillin plus amikacin (Farzana & Shamsuzzaman 2015; Fujimura et al. 2009), and amikacin plus imipenem (Farzana & Shamsuzzaman 2015) have shown good synergistic effects against imipenem-resistant *P. aeruginosa* (IRPA) strains. The aim of this study was to determine the *in vitro* activity of 13 antimicrobial combinations against imipenem-resistant *P. aeruginosa* isolated from patients at Songklanagarind Hospital in Southern Thailand.

Materials and Methods

BACTERIAL ISOLATES

Thirty-eight IRPA isolates were collected from clinical samples such as sputum (27), pus (4), body fluid (3), tissue (3) and blood (1) from patients at Songklanagarind Hospital in Songkhla Province, Thailand during the July 2012-October 2013 period. The bacterial isolation and identification were performed using standard laboratory methods (Giligan 1995). Imipenem-resistant *P. aeruginosa* (IRPA) was defined as an isolate confirmed to be resistant to imipenem. MICs of 8-32 µg/mL were considered as low-level resistance, and MICs greater than 32 µg/mL were considered as high-level resistance (Patzer & Dzierzanowska 2007). The Ethics Committee of the Faculty of Medicine, Prince of Songkla University approved the study (REC 58-268-01-8).

ANTIMICROBIAL SUSCEPTIBILITY TESTING AND MIC DETERMINATION

The antimicrobial susceptibilities and minimal inhibitory concentrations (MICs) of amikacin (0.016-256 µg/mL, AK), aztreonam (0.016-256 µg/mL, ATM), ceftazidime (0.016-256 µg/mL, CAZ), ciprofloxacin (0.002-32 µg/mL, CIP), colistin (0.016-256 µg/mL, CS), imipenem (0.002-32 µg/mL, IMI), and piperacillin/tazobactam (0.016-256 µg/mL, TZP) (Liofilchem, Roseto degli Abruzzi, Italy) were determined using the E-test. The antimicrobial susceptibility and MIC breakpoints for *P. aeruginosa* followed the Clinical and Laboratory Standards Institute (CLSI) guidelines CLSI 2014). *P. aeruginosa* ATCC 27853 was used as the control strain.

ANTIMICROBIAL COMBINATION TESTING

The antimicrobial combination testing was performed following the E-test method (Sueke et al. 2010) by placing E-test strips of the two antimicrobial agents on an inoculated Mueller Hinton agar plate at a 90° angle intersecting at the respective MICs for the organism. The plates were incubated at 35°C to 37°C for 18 h. The inhibition zone of each antimicrobial agent intersecting the E-test strip was interpreted as the MIC of the combination. The fractional inhibitory concentrations (FICs) were calculated by dividing the MIC of drugs A and B in combination by the MIC of drugs A and B alone. The fractional inhibitory concentration index (FICI) was obtained by the sum of the FICs of each drug. FICI values of ≤0.5 were considered synergistic, >0.5 to <4.0 no interaction, and ≥4.0 antagonistic (Fujimura et al. 2009).

Results

SOURCE OF IRPA ISOLATES

We found that the most common sources of IRPA isolates were respiratory tract infections such as ventilator associated pneumonia, pneumonia, and chronic lung disease (74%, 28/38 cases) followed by surgical site infections (16%, 6/38 cases), bloodstream infections (8%, 3/38 cases), and urinary tract infections (3%, 1/38 cases) (Data not shown).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Of the 38 IRPA isolates, the susceptibility rate was highest against colistin, followed by piperacillin/tazobactam, amikacin, ciprofloxacin, ceftazidime, and aztreonam. The MIC_{50} values of amikacin, colistin, ciprofloxacin, and piperacillin/tazobactam were lower than the CLSI susceptibility breakpoint (Table1).

High-level IRPAs were found in 29/38 (76%) of the cases. Colistin exhibited excellent activity against the majority of high-level IRPA isolates (90% susceptibility rates and an MIC_{50} of 1.5 µg/mL). However, colistin-resistant isolates (having MICs 3 µg/mL) were found in 3/29 (10%) of the high-level IRPA isolates. Regarding the other antimicrobials tested, the high-level IRPA isolates showed high rates of susceptibility (52% and 72%) only for amikacin and piperacillin/tazobactam (Table 1).

Low-level IRPAs were found in 9/38 (24%) of the cases. Colistin exhibited excellent activity against all low-level IRPA isolates (100% susceptibility rates and an MIC_{50} of 1.5 µg/mL). Regarding all other antimicrobials tested, the low-level IRPA isolates exhibited high rates of susceptibility (78%-89%) and the MIC_{50} values of all other antimicrobial agents were lower than the CLSI susceptibility breakpoint (Table 1).

TABLE 1. The results of antimicrobial susceptibility testing of 38 imipenem-resistant *P. aeruginosa* (IRPA) isolates against 6 antimicrobial agents

Antimicrobial agent	IRPA (*n*=38)		Antimicrobial susceptibility			
			Low-level IRPA (*n*=9) (MIC_{IMI} of 8-32 μg/mL)		High-level IRPA (*n*=29) (MIC_{IMI} of >32 μg/mL)	
	MIC_{50} (μg/mL)	S (%)	MIC_{50} (μg/mL)	S (%)	MIC_{50} (μg/mL)	S (%)
AK	6	23(60)	3	8(89)	16	15(52)
ATM	12	16(42)	4	7(78)	16	9(31)
CAZ	12	17(45)	2	8(89)	96	9(31)
CS	1.5	35(92)	1.5	9(100)	1.5	26(90)
CIP	0.25	22(58)	0.125	8(89)	6	14(48)
TZP	8	29(76)	2	8(89)	12	21(72)

Amikacin=AK; aztreonam=ATM; ceftazidime=CAZ; colistin=CS; ciprofloxacin=CIP; imipenem=IMI; piperacillin/tazobactam=TZP
MIC breakpoints of AK: susceptible ≤16 μg/mL; ATM: susceptible ≤8 μg/mL; CAZ: susceptible ≤8 μg/mL; CS: susceptible ≤2 μg/mL; CIP: susceptible ≤1 μg/mL and TZP: susceptible ≤16/4 μg/mL (CLSI 2014)
S=susceptible

ANTIMICROBIAL COMBINATIONS TESTING

Of the 38 IRPA isolates, the top five combinations exhibiting high synergistic effects were ceftazidime plus ciprofloxacin (26%, 10/38 isolates), imipenem plus aztreonam (18%, 7/38 isolates), ceftazidime plus amikacin (13%, 5/38 isolates), imipenem plus ciprofloxacin and piperacillin/tazobactam plus aztreonam (10%, 4/38 isolates for both combinations), and piperacillin/tazobactam plus amikacin (5%, 2/38 isolates) - no antagonism was observed in these combinations except for imipenem plus aztreonam (29%, 11/38 isolates) and ceftazidime plus amikacin (3%, 1/38 isolates). Overall, we observed that all combinations of piperacillin/tazobactam with each antimicrobial agent showed synergistic effects with no antagonism (Table 2). Of the 29 high-level IRPA isolates, the top five combinations exhibiting high synergistic effects were imipenem plus aztreonam (24%, 7/29 isolates), ceftazidime plus ciprofloxacin (21%, 6/29 isolates), ceftazidime plus amikacin (17%, 5/29 isolates), piperacillin/tazobactam plus aztreonam (14%, 4/29 isolates), and imipenem plus ciprofloxacin and piperacillin/tazobactam plus amikacin (7%, 2/29 isolates for both combinations) - no antagonism was observed in these combinations except for imipenem plus aztreonam (10%, 3/29 isolates) and ceftazidime plus amikacin (3%, 1/29 isolates). Overall, we observed that all combinations of piperacillin/tazobactam with each antimicrobial agent presented synergistic effects with no antagonism (Table 2).

Synergy was observed in none of the three colistin-resistant isolates. However, antagonism was observed in amikacin plus ciprofloxacin, ceftazidime plus amikacin, ceftazidime plus aztreonam, and imipenem plus amikacin (33%, 1/3 isolates for all combinations) (Data not shown).

Discussion

In our study, we found that colistin remained the most effective antimicrobial agent against IRPA isolates (including both the low-level and high-level IRPA isolates) - 90%-100% susceptibility rates and MIC_{50}s of 1.5 μg/mL. Previous studies have reported similar data - 95%-100% of IRPA isolates being susceptible to colistin and MIC_{50}s of ≤1.5 μg/mL (Nazli et al. 2015; Sanal et al. 2016; Tam et al. 2010). Colistin is recommended for the treatment of multidrug-resistant *P. aeruginosa* infections. However, the associated adverse effects (nephrotoxicity and neurotoxicity) and colistin-resistant strains remain a concern (Dalfino et al. 2015; Leung et al. 2008; Memar et al. 2016).

Our results demonstrated that piperacillin/tazobactam and amikacin are effective antimicrobial agents against high-level IRPA isolates. However, piperacillin/tazobactam and amikacin alone have been associated with a risk of selection for resistant strains (Harris et al. 2002; Kanj & Kanafani 2011), and amikacin alone is recommended only for the treatment of lower urinary tract infections (Kanj & Kanafani 2011; Moore & Flaws 2011).

This study found that amikacin, aztreonam, ceftazidime, ciprofloxacin, and piperacillin/tazobactam were effective antimicrobial agents against low-level IRPA isolates (78%-89% susceptibility rates and low MIC_{50}s), suggesting that these antimicrobial agents could be useful for the treatment of infections due to IRPA isolates, when MICs of imipenem are 12-16 μg/mL. Resistance to imipenem in *P. aeruginosa* is mostly mediated by OprD loss (Pai et al. 2001). This study found 7 of 9 low-level IRPA isolates exhibited only resistance to imipenem. We assume the loss of the OprD porin plays an important role in the majority of these isolates although we did not study the mechanisms of resistance to imipenem in these isolates. However, this possibility should be further evaluated with a larger number of organisms and investigations into the mechanisms of resistance to imipenem.

Our study found that IRPA isolates were the predominant pathogens causing respiratory tract infections, especially ventilator associated pneumonia. A previous study reported that *P. aeruginosa* ventilator associated pneumonia was associated with biofilm formation, which lead to persistent or recalcitrant to antimicrobial agents. Drugs with an MIC were not effective against these isolates. The concentrations of antimicrobials required to kill biofilm isolates under either *in vitro* or *in vivo* conditions can be in excess of 200 times the MIC (Soboh et al. 1995). These findings are in agreement with another study on patients with biofilm infections, in which high dosages of antimicrobial agents within the safe ranges of renal and hepatic functions were suggested (Wu et al. 2015).

P. aeruginosa is an opportunistic human pathogen commonly found in the environment in soil, water, and animals. Because of different intrinsic or acquired mechanisms of resistance, *P. aeruginosa* infections are difficult to treat. We found that colistin, amikacin, ciprofloxacin, and piperacillin/tazobactam were effective antimicrobial agents against the IRPA isolates from our hospital. These isolates were resistant to aztreonam and ceftazidime. A study by Golle et al. (2017) found a high percentage of imipenem-resistant *P. aeruginosa* isolates from clinical (36%, 47/130 isolates) and environmental (52%, 32/61 isolates) settings. Their IRPA isolates showed high percentages of resistance to piperacillin/tazobactam (52%) and ceftazidime (42%) among the clinical isolates and ceftazidime (37%) and ciprofloxacin (35%) among the environmental isolates. The IRPA isolates from the clinical settings showed various antimicrobial resistance patterns, including resistance to carbapenems only and resistance to all classes of antimicrobials tested, while most of the IRPA isolates from environmental settings showed predominant resistance to carbapenems only and resistance to all classes of antimicrobials tested. The data from our results and the study of Golle et al. (2017) indicate that IRPA could be ubiquitous in both clinical and environmental settings, and antimicrobial resistance is a serious concern.

TABLE 2. The results of 13 antimicrobial combinations against 38 imipenem-resistant *P. aeruginosa* (IRPA) isolates

Antimicrobial combination	Interpretation (%)						
	IRPA (*n*=38)	Low-level IRPA (*n*=9) (MIC_{IMI} of 8-32 μg/mL)			High-level IRPA (*n*=29) (MIC_{IMI} of >32 μg/mL)		
	Synergy (%)	Synergy (%)	No interaction(%)	Antagonism (%)	Synergy (%)	No interaction(%)	Antagonism (%)
AK+CIP	0(0)	0(0)	9(100)	0(0)	0(0)	28(96)	1(3)
CAZ+AK	5(13)	0(0)	9(100)	0(0)	5(17)	23(79)	1(3)
CAZ+CIP	10(26)	4(44)	5(56)	0(0)	6(21)	23(79)	0(0)
CAZ+ATM	1(3)	0(0)	9(100)	0(0)	1(3)	27(93)	1(3)
CS+CAZ	1(3)	0(0)	9(100)	0(0)	1(3)	28(96)	0(0)
CS+IMI	0(0)	0(0)	5(56)	4(44)	0(0)	29(100)	0(0)
CS+TZP	0(0)	0(0)	9(100)	0(0)	0(0)	29(100)	0(0)
IMI+AK	1(3)	0(0)	3(33)	6(67)	1(3)	27(93)	1(3)
IMI+CIP	4(10)	2(22)	7(78)	0(0)	2(7)	27(93)	0(0)
IMI+ATM	7(18)	0(0)	1(11)	8(89)	7(24)	19(65)	3(10)
TZP+AK	2(5)	0(0)	9(100)	0(0)	2(7)	27(93)	0(0)
TZP+CIP	1(3)	0(0)	9(100)	0(0)	1(3)	28(96)	0(0)
TZP+ATM	4(10)	0(0)	9(100)	0(0)	4(14)	25(86)	0(0)

Aztreonam=ATM; amikacin=AK; ceftazidime=CAZ; colistin=CS; ciprofloxacin=CIP; imipenem=IMI; piperacillin/tazobactam=TZP

In this study, the synergy rates in combinations of β-lactams and aminoglycosides, β-lactams and fluoroquinolones, β-lactams and aztreonam, colistin and β-lactams or piperacillin/tazobactam, piperacillin/tazobactam and aminoglycosides or fluoroquinolones or aztreonam, and aminoglycosides and fluoroquinolones against IRPA isolates were 3%-13%, 10%-26%, 3%-18%, 0%-3%, 3%-10%, and 0%, respectively.

Synergy from these combinations has been reported in many studies and higher or lower synergy rates for the same combinations against IRPA isolates have been found by different investigators (Amer & Abd-Elmonsef 2016; Dundar & Otkun 2010; Farzana & Shamsuzzaman 2015; Sader et al. 2003; Song et al. 2003; Tellis et al. 2016; Yasmin et al. 2013). However, the comparison of results from different studies is difficult due to variations in microbiology tests, methods, and definitions of synergy among studies (Sader et al. 2003). Of note, we used the e-test method due to it is simple to use in routine clinical practice, time-efficient, inexpensive, and has been shown to be useful for performing *in vitro* synergy testing in *P. aeruginosa* and other bacterial species (Pankey & Ashcraft 2005; White et al. 1996).

Regarding combinations of β-lactams and aminoglycosides, we found that ceftazidime plus amikacin exhibited synergy rates against IRPA and high-level IRPA isolates of 13%-17% and low levels of antagonism (3% for both IRPA and high-level IRPA isolates). Previously, Song et al. (2003) found that the combination of ceftazidime plus amikacin produced a 17% rate of synergy against 24 IRPA isolates, with no antagonism detected (Song et al. 2003). Likewise, Nazli et al. (2015) reported that the synergy rate of ceftazidime plus amikacin against 60 *P. aeruginosa* isolates, of which some isolates were resistant to imipenem, was 15%, with no antagonism detected in this combination (Nazli et al. 2015). Thus, ceftazidime plus amikacin could be useful in combatting IRPA isolates. However, combining a 3rd or 4th generation cephalosporin along with amikacin should be considered as the first choice of therapy when the patient has no other factors predisposing to nephrotoxicity (Tellis et al. 2016). Looking at imipenem plus amikacin against IRPA isolates, various synergy rates have been reported by earlier investigators, with no antagonism detected (Farzana & Shamsuzzaman 2015; Nazli et al. 2015; Song et al. 2003). We found that imipenem plus amikacin exhibited less synergy against IRPA and high-level IRPA isolates (3% for both IRPA and high-level IRPA isolates) - antagonism was also discovered in 18% of IRPA and 3% of high-level IRPA isolates. In addition, we also found that all IRPA isolates in which antagonism effects were detected were susceptible to amikacin (MICs of 1.5-4 μg/mL), which suggests that antagonism may occur between two antimicrobial agents although the isolates are susceptible to one antimicrobial agent and considering that imipenem plus amikacin may not be the most suitable choice to treat IRPA infections. In agreement with another study, we suspect that antagonism effects in IRPA isolates might be influenced by the mechanism of resistance to imipenem (Pai et al. 2001); imipenem cannot enhance the uptake of amikacin.

Among the combinations of β-lactams and fluoroquinolones evaluated, synergy or indifference have generally been detected with antagonism only occasionally being reported in small percentages of IRPA isolates (Altoparlak et al. 2005; Dundar & Otkun 2010; Song et al. 2003; Tellis et al. 2016). A previous study by Altoparlak et al. (2005) showed good synergistic effects of ceftazidime plus ciprofloxacin against 7 of 21 (33%) IRPA isolates which were resistant to ceftazidime and ciprofloxacin separately (Altoparlak et al. 2005). In this study, we found that ceftazidime plus ciprofloxacin displayed the highest percentage (26%, 10/38 isolates) of synergy against IRPA isolates and a high percentage (21%, 6/29 isolates) of synergy against high-level IRPA isolates, with no antagonism detected. In addition, we also found that 8 of 10 IRPA isolates and 4 of 6 high-level IRPA isolates in which synergistic effects were detected were susceptible to ceftazidime (MIC ranges of 1.5-4 μg/mL) and ciprofloxacin (MIC ranges of 0.094-0.75 μg/mL). The other IRPA isolates were high-level IRPAs (1 of 2 isolates was resistant to ceftazidime (MIC of 12 μg/mL), but was susceptible to ciprofloxacin (MIC of 0.25 μg/mL) and 1 of 2 isolates was resistant to both ceftazidime (MIC of >256 μg/mL) and ciprofloxacin (MIC of >32 μg/mL). These results suggest that ceftazidime plus ciprofloxacin could be useful for the treatment of infections due to IRPA isolates including high-level IRPAs, although the isolate exhibited resistance to ceftazidime and ciprofloxacin. Furthermore, we observed that imipenem plus ciprofloxacin yielded synergy rates against these isolates of high-level IRPAs and IRPA isolates of 7-10% with - no antagonism in this combination. Our findings indicate that these combinations could be choices for IRPA isolates and may be another alternative to replace β-lactams plus aminoglycosides, when the physician faces limitations due to the toxicity of aminoglycosides (Song et al. 2017). In future studies, it will be necessary to determine the synergistic effects of ceftazidime plus ciprofloxacin against a larger number of IRPA isolates resistant to ceftazidime and ciprofloxacin.

Combinations of piperacillin/tazobactam plus either amikacin or ciprofloxacin or aztreonam have been reported to show synergistic results (14%-96%) against IRPA isolates (Altoparlak et al. 2005; Amer & Abd-Elmonsef 2016; Farzana & Shamsuzzaman 2015; Fujimura et al. 2009). However, antagonism may occur in the combination of piperacillin/tazobactam plus ciprofloxacin (Fujimura et al. 2009). In our study, we observed that the combinations of piperacillin/tazobactam plus amikacin or ciprofloxacin or aztreonam showed 3-10% synergy against IRPA and 3-14% synergy against high-level IRPA isolates, with no antagonism detected. Although our results had less synergy in the tested combinations against IRPA isolates than other investigations, we observed no antagonisms in these

combinations. This suggests that piperacillin/tazobactam plus amikacin or ciprofloxacin or aztreonam could be promising alternatives for fighting IRPA and high-level IRPA isolates. For the mechanism of the synergy of these combinations, we speculate that piperacillin/tazobactam may enhance the uptake of other antimicrobial agents by the bacterial cells, because piperacillin inhibits cell wall synthesis (Fujimura et al. 2009).

Although amikacin plus ciprofloxacin has been reported to have synergistic or additive effects against *P. aeruginosa* (Gerceker & Gurler 1995; Yasmin et al. 2013), our study indicates that this combination may not be useful for IRPA and high-level IRPA isolates, as we observed no synergy with amikacin plus ciprofloxacin in all of the IRPA and high-level IRPA isolates tested and we also found antagonism in some isolates.

Aztreonam has been combined with other antimicrobial agents, as it has been found to enhance the antimicrobial spectrum of co-drugs and yield potential synergistic results (Sader et al. 2003). In our study, a high synergy potential was found in imipenem plus aztreonam when tested against high-level IRPA isolates (24%); however, antagonism also occurred in 10% of the isolates. Of note, imipenem has been reported to be a strong inducer of class I beta-lactamase production (Tausk et al. 1985). It is possible that the mechanism of the antagonism effect in this combination may have been a result of inhibited aztreonam potency by imipenem induced beta-lactamase production in the tested isolates (Yamaki et al. 1998).

In our study, the combinations of colistin and β-lactams (ceftazidime or imipenem or piperacillin/tazobactam) exhibited low synergy rates (0-3%) against the IRPA and high-level IRPA isolates tested and also antagonism was observed in 10% of the colistin plus imipenem against IRPA isolates. A recent study on colistin plus either ceftazidime or imipenem also reported no synergy against IRPA isolates (Nazil et al. 2015), indicating that combinations of colistin and β-lactams, especially colistin plus imipenem, may not represent potential options for IRPA cases - even though another investigation found 50% synergy of colistin plus imipenem against these isolates (Farzana & Shamsuzzaman 2015). The mechanism of colistin in combination with other antimicrobials is to increase permeabilization of the outer membrane, allowing other antimicrobials to enter the bacterial cells (Sanal et al. 2016). We suspect that the cause of lower rates of synergy or antagonism in these combinations against our isolates may have been a result of colistin's inability to enhance the uptake of β-lactams.

A recent study suggested that the increasing use of colistin for the treatment of infections caused by *P. aeruginosa* may lead to the emergence of colistin-resistant strains (Memar et al. 2016). This study was based on data from several countries, finding prevalence rates of *P. aeruginosa* resistance to colistin from 2 to 30% (Memar et al. 2016). In our study, we found that 8% of the IRPA isolates were resistant to colistin. In addition, the study of Vidaillac et al. (2012) found that colistin with other antimicrobials had no activity against colistin-resistant strains (Vidaillac et al. 2012). In our study, all tested combinations showed no synergy against colistin-resistant isolates and antagonism also occurred in 33% of the isolates for amikacin plus ciprofloxacin, ceftazidime plus amikacin, ceftazidime plus aztreonam, and imipenem plus amikacin, indicating that these combinations may not be an effective option against infections caused by colistin-resistant isolates with antagonism effects. Of note, a previous study reported colistin-resistant *P. aeruginosa* showed overexpression of the MexAB-OprM and MexXY-OprM efflux pumps, which are associated with cross-resistance to different antimicrobial agents including aminoglycosides, β-lactams, and fluoroquinolones (Goli et al. 2016). Consequently, it is possible that overexpression of these efflux pumps might have been associated with an antagonism effect in these combinations against our isolates.

The limitations of this study were the small number of isolates, the lack of testing of mechanisms of resistance to antimicrobial agents in IRPA isolates, especially acquired resistance mechanisms (i.e. mutations in the specific porin OprD reduce the uptake of imipenem, the overexpression of MexXY-OprM owing to mutations in genes encoding *mexZ* leads to aminoglacoside, fluoroquinolone and cefepime resistance in clinical strains of *P. aeruginosa*, and mutations in *gyrA* and alterations in two efflux systems (MexCD-OprJ and MexEF-OprN) leading to ciprofloxacin resistance) (Breidenstein et al. 2011), and our inability to explain the mechanisms responsible for the antimicrobial interactions investigated. Further studies investigating these mechanisms with larger numbers of isolates are needed to obtain more authoritative data.

Conclusion

In summary, among all of the antimicrobials tested, we found that colistin had the greatest level of activity against most IRPA isolates. Ceftazidime plus ciprofloxacin displayed useful properties in treating infections caused by IRPA, including high-level IRPA isolates, while ceftazidime plus amikacin, imipenem plus ciprofloxacin, and combinations of piperacillin/tazobactam plus amikacin or aztreonam showed promise as alternative options for treatment of IRPA cases. Colistin-resistant isolates were detected in 3 (10%) of the high-level IRPAs and all of the antimicrobial combinations tested against this isolate produced no synergy. Antagonism was found in amikacin plus ciprofloxacin, ceftazidime plus amikacin, ceftazidime plus aztreonam, and imipenem plus amikacin, thus colistin-resistant isolates need to be closely monitored.

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