Activity of Amikacin, Ertapenem, Ciprofloxacin and Levofloxacin alone and in Combination against Resistant Nosocomial Pathogens by Time-Kill

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ABSTRACT

Objective: *The purpose of this study was to determine the synergistic activity of amikacin/ertapenem, fluoroquinolones (ciprofloxacin and levofloxacin)/ertapenem and amikacin/fluoroquinolones combinations against resistant nosocomial pathogens.*

Methods: *Time-kill studies were performed over 24 hours using an inoculum of 5 x 10^6 – 1 x 10^7 cfu/mL. Antibiotics were tested at the 1 x MIC and 4 x MIC concentrations.*

Results: *At MIC and/or 4 x MIC concentrations, the antibiotic combinations showed additive or synergistic activity against* Acinetobacter *strains and extended spectrum beta-lactamase producing* Klebsiella pneumoniae. In Escherichia coli *strains, synergy was seen when amikacin was combined with ertapenem, ciprofloxacin and levofloxacin; ertapenem in combination with fluoroquinolones demonstrated antagonism. In* Pseudomonas aeruginosa *strains, synergistic effect was exhibited by ertapenem plus amikacin and ertapenem plus fluoroquinolones. The antibiotic combinations showed antagonistic interaction in methicillin-resistant* Staphylococcus aureus *and vancomycin-resistant* Enterococcus faecalis.

Conclusion: *The antibiotic combinations showed additive or synergistic activity against many gram-negative pathogens.*

Actividad Letalidad-tiempo de la Amicacina, la Ertapenema, la Ciprofloxacina y la Levofloxacina, Solas o en Combinación, Frente a los Patógenos Nosocomiales

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RESUMEN

Objetivo: *El propósito del presente estudio fue determinar la actividad sinérgica de la amicacina/ ertapenema/fluoroquinolonas (ciprofloxacina y levofloxacina)/ertapenema y amicacina/y combinaciones de fluoroquinolonas frente a patógenos nosocomiales resistentes.*

Métodos: *Se realizaron estudios de letalidad-tiempo por 24 horas, usando un inóculo de 5 x 10^6 – 1 x 10^7 cfu/mL. Se probaron antibióticos en concentraciones de 1xCIM y 4xCIM.*

Resultados: *En concentraciones de CIM y/o 4 x CIM, las combinaciones de antibióticos mostraron actividad aditiva o sinergésica frente a las cepas* Acinetobacter y Klebsiella pneumoniae *productoras de la beta-lactamasa de espectro extendido. En las cepas de* Escherichia coli, *se observó sinergia cuando se combinó la amicacina con la ertapenema, la ciprofloxacina y la levofloxacina; la ertapenema en combinación con las fluoroquinolonas demostró antagonismo. En las cepas de* Pseudomonas aeruginosa, *se puso de manifiesto un efecto sinergésico al sumar la ertapenema con amicacina y la ertapenema con fluoroquinolonas. Las combinaciones antibióticas mostraron interacción antagonística en* Staphylococcus aureus *resistente a la meticilina y Enterococcus faecalis resistente a la vancomicina.*

Conclusión: *Las combinaciones antibióticas mostraron actividad aditiva o sinergésica frente a muchos patógenos gram-negativos.*

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INTRODUCTION

Nosocomial pathogens commonly cause severe infections in clinical practice especially in high risk populations such as oncology, transplant and intensive care unit patients (1–3). Extended spectrum beta-lactamase (ESBL) producing *Escherichia coli*, ESBL producing *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp and methicillin-resistant *Staphylococcus aureus* (MRSA) are the most frequently encountered pathogens in these wards (3–7). Nosocomial infections caused by these bacteria are frequently difficult to eradicate using a single antimicrobial agent because of the ability of bacteria to develop resistance. Therefore, the general recommendation is to use combination therapy. Generally, the combination of aminoglycosides with beta-lactams has been used in therapy. Today, fluoroquinolones are often considered as less nephrotoxic alternatives to aminoglycosides. Ciprofloxacin and levofloxacin are the most frequently used fluoroquinolones in combination therapy (1, 2, 5).

Ertapenem, a new carbapenem, has limited activity against nosocomial pathogens such as *P aeruginosa*, *Acinetobacter* spp, MRSA and Enterococci. When ertapenem is combined with other agents, the antimicrobial spectrum of ertapenem may expand (8). Combination therapy is used to obtain synergistic activity, prevent the emergence of resistant mutants during therapy, minimize toxicity and expand the antimicrobial spectrum (8–12).

The purpose of the present study was to determine the synergistic activity of amikacin/ertapenem, fluoroquinolones (ciprofloxacin and levofloxacin)/ertapenem and amikacin/fluoroquinolones combinations against resistant nosocomial pathogens.

MATERIALS AND METHODS

Bacterial strains

The test organisms isolated from hospitalized patients included two ESBL (+) *E. coli*, two ESBL (+) *K pneumoniae*, two *P aeruginosa*, two *Acinetobacter* spp., two *Mec A* (+) MRSA and two Van A genotype vancomycin-resistant *Enterococcus faecalis* (VRE).

Antibiotics and determination of MICs

Amikacin (Eczacýbaþý, Istanbul, Turkey), ertapenem (Merck Sharp & Dohme, Istanbul, Turkey), levofloxacin (Fako, Istanbul, Turkey) and ciprofloxacin (Koçak, Istanbul, Turkey) were provided by the manufacturers. MIC levels of antibiotics were determined by the microdilution method using Mueller-Hinton broth (MHB) (Merck, Darmstadt, Germany) according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) (13). *Escherichia coli* ATCC 25922 was used as the reference strain.

Time-kill curve studies

For each strain, antibiotics were studied alone and in combination with other antibiotics at the MIC and 4 x MIC concentrations. In accordance with the study of Pillai SK *et al* (14), time-kill studies were performed in flasks containing MHB and single or combinations of antibiotics. Overnight bacterial cultures were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard, and further diluted to yield a starting inoculum ranging between 5 x 10^6 – 1 x 10^7 cfu/mL. In each case, an antibiotic-free control was prepared and the same procedure applied. At 0, 6 and 24-hour of incubation at 37°C, samples were removed from test and growth-control cultures and appropriately diluted with cold 0.9% of sodium chloride and inoculated onto Mueller-Hinton agar (Merck, Darmstadt, Germany) plates. After incubation at 37°C for 24 or 48 hours, bacterial colonies were counted. All time-kill studies were performed twice.

Synergy or antagonism was defined as a 100-fold increase or decrease in bacterial colony count compared to the effect of the single most active agent at 24 or 48 hours.

RESULTS

Antibiotic MICs for strains were shown in Table 1. All strains were resistant to fluoroquinolones. Except VRE and *Acinetobacter* strains, all strains were susceptible to amikacin. Whilst *E coli* and *K pneumoniae* were found to be susceptible to ertapenem, MRSA strains were found to be resistant. *Pseudomonas aeruginosa* and *Acinetobacter* strains showed increased MICs of ertapenem. Ertapenem MICs of VRE strains were determined as 1 and 8 mg/mL.

Table 1: MIC values of antibiotics against the test strains

	MIC (µg/mL)			
Strain	**Amikacin**	**Ertapenem**	**Ciprofloxacin**	**Levofloxacin**
E coli 1*	0.25	0.015	16	8
E coli 2*	8	0.03	32	4
K pneumoniae 1*	8	0.125	64	32
K pneumoniae 2*	2	2	64	16
P aeruginosa 1	8	64	2	4
P aeruginosa 2	8	64	2	4
Acinetobacter 1	64	16	64	4
Acinetobacter 2	64	16	64	8
MRSA 1	4	32	16	8
MRSA 2	8	16	4	8
VRE 1	32	1	16	16
VRE 2	32	8	16	16

* ESBL producing microorganisms

Time-kill studies

In *E coli* strains, amikacin plus ertapenem (1 x MIC) and amikacin with ciprofloxacin and levofloxacin (4 x MIC) combinations showed synergistic effect. Ertapenem in combination with fluoroquinolones demonstrated antagonism in these strains. Synergistic effect was seen with amikacin plus ertapenem and levofloxacin and also ertapenem plus ciprofloxacin at MIC concentrations in *K pneumoniae* strains. At 4 x MIC concentrations, amikacin with ciprofloxacin and ertapenem with ciprofloxacin and levofloxacin

demonstrated synergistic interaction in the same isolates (Figure 1, 2). Synergistic effect was detected in all antibiotic combinations (4 x MIC) in *Acinetobacter* spp. In *P aeruginosa* strains, synergistic effect was exhibited between ertapenem and ciprofloxacin (1 x MIC), amikacin and ertapenem (4 x MIC) and ertapenem and levofloxacin (4 x MIC). In MRSA and VRE strains, antagonistic interaction was observed between test antibiotics. Additive synergistic and antagonistic interactions determined by time-kill tests are summarized in Table 2.

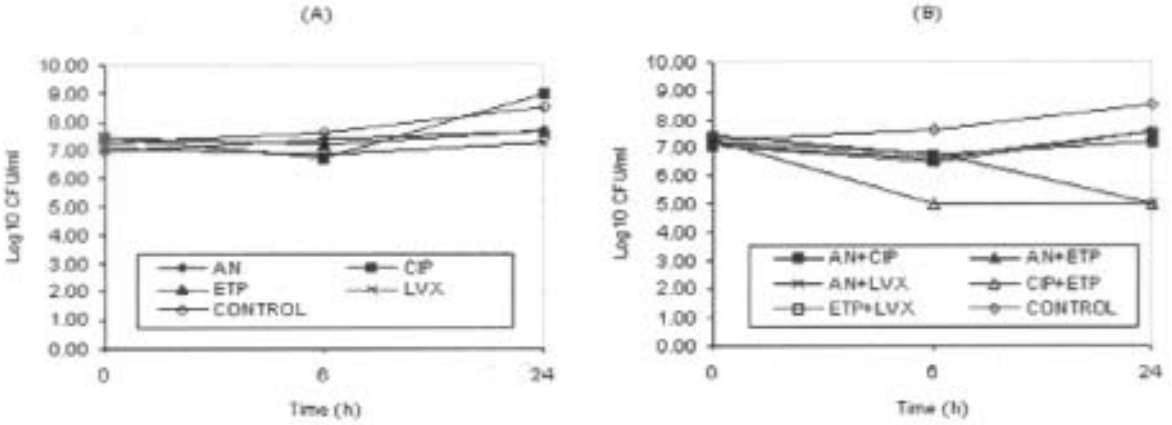

Fig. 1: Time-kill curves of *K pneumoniae* 2 strain exposed to anti-microbial agents at the MIC concentration. AN: Amikacin, ETP: Ertapenem, CIP: Ciprofloxacin, LVX Levofloxacin. A: u AN alone, s ETP alone, n CIP alone, **x** LVX alone, p control. B: n AN+CIP (additive interaction), **x** AN+LVX (synergistic inter-action), ~ ETP + LVX (indifference), s AN + ETP (indifference), V CIP + ETP (synergistic interaction), p control.

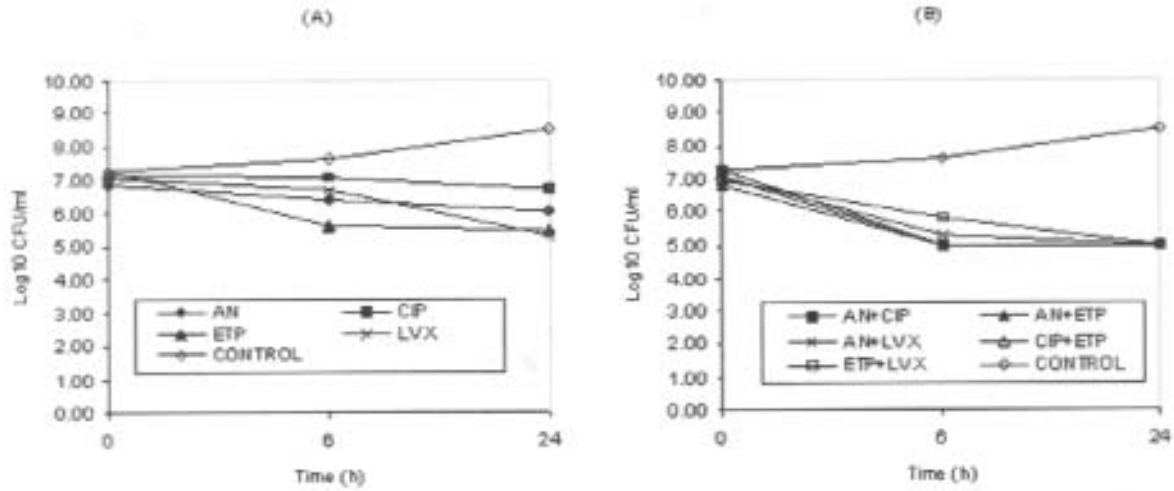

Fig. 2: Time-kill curves of *K pneumoniae* 2 strain exposed to antimi-crobial agents at the 4 x MIC concentration. AN: Amikacin, ETP: Ertapenem, CIP: Ciprofloxacin, LVX Levofloxacin. A: u AN alone, s ETP alone, n CIP alone, **x** LVX alone, p control. B: n AN+CIP (synergistic interaction), **x** AN+LVX (additive interaction), ~ ETP + LVX (indifference), s AN+ETP (indifference), V CIP + ETP (indifference), p control.

Therapy involving an aminoglycoside plus a beta-lactam has for many years represented the therapy of choice for treatment of infections caused by nosocomial Gram-negative pathogens. Today, fluoroquinolones are used as a reasonable alternative to aminoglycosides for treating these infections**.** Advantages of fluoroquinolones include less nephrotoxicity and oral administration (4, 15).

As we considered the effectiveness of antibiotic combinations against gram-negative bacteria in this study, it was seen that most of the antibiotic combinations showed additive or synergistic activity in ESBL producing *K pneumoniae* and *Acinetobacter* strains. In *E coli* strains, synergy was seen

DISCUSSION

Severe Gram-positive and Gram-negative infections, particularly in compromised hosts, require aggressive empiric therapy with at least two antimicrobial agents. Combinations of agents that exhibit synergy or partial synergy could potentially reduce toxicity and improve outcome for patients with infections that are difficulty to treat (1, 11).

Table 2: Antibiotic interactions by time-kill studies

Antibiotic combinations	MIC		4 x MIC	
	6h	24h	6h	24h
Amikacin + Ertapenem				
E coli 2		Synergistic		
K pneumoniae 1		Synergistic		
Acinetobacter 1				Synergistic
P aeruginosa 1				Synergistic
P aeruginosa 2				Synergistic
MRSA 2		Antagonistic		Additive
MRSA 1				Antagonistic
Amikacin + Levofloxacin				
E coli 1			Synergistic	Synergistic
E coli 2		Additive		
K pneumoniae 1		Additive		
K pneumoniae 2		Synergistic	Additive	
Acinetobacter 1	Additive	Additive		Synergistic
Acinetobacter 2	Additive	Additive		
P aeruginosa 1		Additive		
MRSA 1			Additive	Additive
VRE 1		Antagonistic		
Amikacin + Ciprofloxacin				
E coli 2				Synergistic
K pneumoniae 1	Additive			
K pneumoniae 2		Additive	Synergistic	Synergistic
Acinetobacter 1			Additive	Synergistic
P aeruginosa 1	Additive	Additive		
MRSA 2		Antagonistic		
MRSA 1			Additive	
Ertapenem + Ciprofloxacin				
E coli 1		Antagonistic		Antagonistic
E coli 2		Additive	Antagonistic	
K pneumoniae 1			Synergistic	
K pneumoniae 2	Synergistic	Synergistic		
Acinetobacter 1				Synergistic
Acinetobacter 2			Additive	Synergistic
P aeruginosa 1	Additive	Synergistic		
P aeruginosa 2		Additive		
MRSA 2	Antagonistic	Antagonistic		
VRE 1				Antagonistic
VRE 2			Additive	Additive
Ertapenem + Levofloxacin				
E coli 1		Antagonistic		
E coli 2		Additive		
K pneumoniae 1		Additive	Additive	Synergistic
Acinetobacter 1				Synergistic
P aeruginosa 2			Synergistic	
VRE 1		Antagonistic		

when amikacin was combined with ertapenem, ciprofloxacin and levofloxacin and ertapenem in combination with fluoroquinolones demonstrated antagonism. In *P aeruginosa* strains, synergistic effect was exhibited by ertapenem plus amikacin and ertapenem plus fluoroquinolones. As for the antibiotic concentrations, a higher rate of synergy was observed in antibiotic combinations at 4 x MIC concentration compared to those at 1 x MIC concentration. In Gram-positive bacteria, antagonism was detected most often with amikacin plus ertapenem and ertapenem plus ciprofloxacin combinations.

Synergistic interaction has been reported between beta-lactams and aminoglycosides in many studies (2, 3, 16, 17). For beta-lactam and fluoroquinolone combinations, variable antimicrobial interactions have been reported. Generally, synergism, additivity or indifference has been demonstrated. Antagonism has occasionally been reported in a small percentage of isolates (1, 9, 15).

By using the checkerboard method, Song *et al* (1) investigated whether beta-lactam/aminoglycoside/fluoroquinolone combinations had synergistic activity against 24 strains of *P aeruginosa* that are resistant to these antibiotics.

While antagonism was not detected in any combinations, synergistic effect was observed in one or more antibiotic combinations in 15 of 24 strains.

In one study (18), synergy was demonstrated by amikacin with fluoroquinolones against all quinolone-susceptible *Acinetobacter* strains. Burgess and Hastings used time-kill method in a study (15) and investigated the *in vitro* efficiency of beta-lactam antibiotics in combination with amikacin and fluoroquinolones against *P aeruginosa* strains. The researchers determined that beta-lactam/amikacin combination demonstrated a higher rate of synergism compared to beta-lactam/fluoroquinolone combination and did not detect antagonism.

In the study of Diez Enciso (19), in which beta-lactam/aminoglycoside and beta-lactam/fluoroquinolone combinations were compared, the author determined that the combinations with aminoglycosides demonstrated a higher rate of synergism. However, there are also results which are in contradiction with this finding (20).

Burgess and Nathisuwan (3) did not determine any difference between combinations of beta-lactam antibiotics with gentamicin, ciprofloxacin and levofloxacin in terms of synergism. In this study, a difference was not determined between beta-lactam/aminoglycoside and beta-lactam/fluoroquinolone combinations which is consistent with the results of the study of Burgess and Nathisuwan.

In a study conducted in 2004 (5), the *in vitro* efficiencies of levofloxacin and ciprofloxacin in combination with beta-lactams and amikacin against *P aeruginosa* and *Acinetobacter* strains were compared. The researchers detected synergy and additive effect in all combinations by using the checkerboard method and they showed signs of synergy in at least one combination in all strains by using the time-kill method. Finally, they did not determine a difference between levofloxacin and ciprofloxacin in terms of synergistic effect.

Isenberg *et al* (21) also detected no difference in the rate of synergy between ciprofloxacin and levofloxacin-based combinations. Similar to the findings of the researchers mentioned above, a difference was not observed in this study between ciprofloxacin and levofloxacin when combined with ertapenem and amikacin in terms of synergism.

It was thought that synergism was obtained only when the organism was susceptible to two antimicrobial agents. However, it was shown by Song *et al* (1) and Cappelletty and Rybak (22) that synergy may occur between two antimicrobial agents although the strains were resistant to the individual antibiotics.

Similar to these studies, we detected synergism in combinations even though both agents were resistant when they were tested alone in Gram-negative isolates.

In conclusion, synergy was more common with amikacin plus ertapenem and ertapenem plus ciprofloxacin combinations in Gram-negative bacteria in this study. In Gram-positive bacteria, synergistic activity was not observed with any antibiotic combinations.

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