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Synergy of drug combinations in treating multidrug-resistant *Pseudomonas aeruginosa*

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RESEARCH

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ABSTRACT

Background

With the emergence of metallo-betalactamases (MBL) in *Pseudomonas aeruginosa (P. aeruginosa),* the value of carbapenem, the drug of last resort, is being severely compromised. Curtailing the use of carbapenems becomes paramount if resistance is to be reined in.

Aims

To study the role of synergy between combinations of drugs as an alternative treatment choice for *P. aeruginosa*. Synergy was studied between combinations of levofloxacin with piperacillin-tazobactam and levofloxacin with cefoperazone-sulbactam by time-kill and chequerboard techniques.

Methods

P. aeruginosa were tested for antibiotic susceptibility by the disc diffusion assay (260 isolates) and E-test (60 isolates). Synergy testing by chequerboard and time-kill assays was performed with combinations of piperacillin-tazobactam with levofloxacin (11 isolates) and cefoperazone-sulbactam with levofloxacin (10 isolates).

Results

Nearly all isolates were susceptible to piperacillin-tazobactam (96.1 per cent), followed by piperacillin (78.5 per cent). Seventy-one isolates (27.3 per cent) were found to be multidrug resistant and 19.6 per cent were ESBL producers. MIC_{50} of amikacin was 32µg/ml and MIC_{90} was 64µg/ml. MIC_{50} and MIC_{90} of cefoperazone-sulbactam was 32µg/ml and 64µg/ml, and for levofloxacin it was 10µg/ml and 240µg/ml, respectively. Piperacillin-tazobactam had MIC_{50} and MIC_{90} of 5µg/ml and 10µg/ml, respectively. Synergy was noted in 72.7 per cent isolates for levofloxacin and piperacillin-tazobactam combination, the remaining 27.3 per cent isolates showed addition by both chequerboard and time-kill assay. For levofloxacin and cefoperazone-sulbactam, only 30 per cent isolates had synergy, 40 per cent showed addition, 20 per cent indifference, and 10 per cent were antagonistic by the chequerboard method.

Conclusion

The combination of levofloxacin and piperacillin-tazobactam is a good choice for treatment of such strains.

Key Words

Multidrug resistance, synergy, time-kill assay, chequerboard technique

What this study adds:

1. What is known about this subject?

Several studies have documented the advantage of synergistic combination of antimicrobials of different groups for treating multidrug-resistant pathogens.

2. What new information is offered in this study?

In this study, levofloxacin with piperacillin-tazobactam emerged as an effective treatment alternative that could be used before carbapenems in the treatment of multidrug-resistant strains.

3. What are the implications for research, policy, or practice?

In multidrug-resistant strains, combination treatment using drugs demonstrating synergy, such as levofloxacin with piperacillin-tazobactam, may be effective in treating patients with multidrug-resistant infections.

Background

Pseudomonas aeruginosa (P. aeruginosa), the most prominent nosocomial pathogen, has intrinsic resistance to many drug classes, along with an ability to acquire resistance to all available treatment options.[1] Primary mechanisms of acquisition of drug resistance include reduced cell permeability, efflux pumps, changes in target enzymes, and inactivation of the antibiotics.[2,3]

No single mutation compromises every antipseudomonal drug. Nevertheless, upregulated efflux systems can simultaneously compromise fluoroquinolones and most ß-lactams, leaving only the aminoglycosides and imipenem (to which mutational resistance evolves at high frequency). The selection of resistant mutants, a risk associated with any antipseudomonal therapy, varies with the type and dosage of antibiotic used and the infection site.[4]

Combination therapy is thus used with the aim of expanding the antimicrobial spectrum, minimising toxicity, preventing the emergence of resistant mutants during therapy, and obtaining synergistic antimicrobial activity.[5,6] The checkerboard titration method and the time-kill curve technique have been the most commonly used methods to determine synergism.[7–9]

Method

The study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Amu, Aligarh, India, between April 2009 and September 2010. Two-hundred-and-sixty strains of *P. aeruginosa* from different sources were subjected to antimicrobial sensitivity testing by the Kirby-Bauer disc diffusion method[10] for the following antimicrobial agents: ceftazidime (30µg), gatifloxacin (5µg), cefepime (30µg), ceftriaxone (30µg), levofloxacin (5µg), cefoperazone (75µg), ceftazidime-clavulanic acid (30/10µg), cefoperazone-sulbactam (75/75µg), ticarcillin (75µg), ticarcillin-clavulanic acid (75/10µg), tobramycin (10µg), amikacin (30µg), piperacillin (100µg), piperacillin-tazobactam (100/10µg), and imipenem (10µg). Isolates resistant to ß-lactams, aminoglycosides, and fluoroquinolones were termed multidrug-resistant isolates. Extended spectrum ß-lactamase (ESBL) production was determined by the disc potentiation method.[11]

Minimum inhibitory concentration (MIC) was estimated for 60 representative isolates of differing levels of drug resistance for three drugs, namely, cefoperazone-sulbactam (Cfs) by E-test (Hi-Media),[10] and for levofloxacin (Le) and piperacillin-tazobactam (Pt) by the standard broth dilution method.[10] Accordingly, these isolates were divided into three groups based on their resistance pattern to different classes of antimicrobials—i.e., aminoglycosides (amikacin), ß-lactams (piperacillin), ß-lactams with inhibitors (cefoperazone–sulbactam), and fluoroquinolones (levofloxacin)—as follows:

- Group 1 consisted of those isolates that were resistant to all four groups of antimicrobials and comprised 10 isolates.
- Group 2 consisted of those isolates that were resistant to any three groups of antimicrobials and comprised 20 isolates.
- Group 3 consisted of those isolates that were resistant to any one or two group of antimicrobials and comprised 30 isolates.

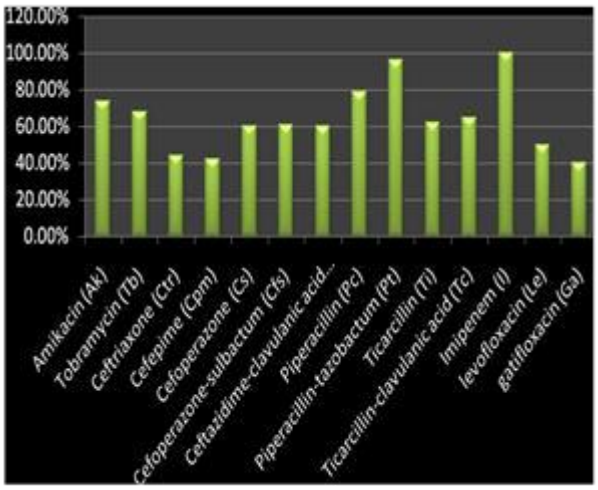
Synergy testing by chequerboard and time-kill assays was performed for two combinations of antimicrobials as follows: piperacillin-tazobactam with levofloxacin (11 isolates), and cefoperazone-sulbactam with levofloxacin (10 isolates). Chequerboard synergy was performed as described previously.[10] Fractional inhibitory concentrations (FICs) were calculated as (MIC of drug A or B in combination) / (MIC of drug A or B alone), and the FIC index was obtained by adding the FIC values. FIC indices were interpreted as synergistic if values were ≤0.5, additive >0.5–1.0, indifferent if >1–2, and antagonistic if >2.0.[10]

Isolates were tested for synergy between levofloxacin and piperacillin-tazobactam and levofloxacin and cefoperazone-sulbactam by time-kill assay as described by Hayami et al.[12] Viable counts were performed at 0, 2, 4, and 24 hours. Concentration of the combined MICs were as follows; ¼A+ ¼B, ¼A+2B, 2A+ ¼B, 2A+2B. Synergy was defined as ≥3 $\log_{10}$ decrease in colony count at 24 hours by the combination compared to the most active single agent. Indifference was taken as <3 $\log_{10}$ increase or decrease in colony count at 24 hours by the combination compared with that by the most active drug alone, and 3 $\log_{10}$ increase in colony count at 24 hours was taken as antagonism.[12]

Results

Antimicrobial susceptibility of *P. aeruginosa* strains from specimens is given in Figure 1. Of the various groups tested, *P. aeruginosa* showed maximum sensitivity to antipseudomonal penicillins with inhibitors (piperacillin-tazobactam: 96.1 per cent, ticarcillin-clavulanic acid: 64.3 per cent), followed by antipseudomonal penicillins alone (piperacillin: 78.5 per cent, ticarcillin: 61.9 per cent). *P. aeruginosa* showed a moderate degree of sensitivity to aminoglycosides (amikacin 73.8 per cent, and tobramycin 68.1 per cent). Cephalosporins with β-lactamase inhibitors (cefoperazone-sulbactam: 60.8 per cent, ceftazidime-clavulanic acid: 60.4 per cent) had better activity against *P. aeruginosa* than plain cephalosporins (cefoperazone: 60.4 per cent, ceftriaxone: 43.8 per cent, and cefepime: 42.3 per cent. Seventy-one isolates (27.3 per cent) were found to be multidrug resistant and 19.6 per cent were ESBL producers. MIC_{50} of amikacin was 32µg/ml and MIC_{90} was 64µg/ml. MIC_{50} and MIC_{90} of cefoperazone-sulbactam was 32µg/ml and 64µg/ml, and for levofloxacin it was 10µg/ml and 240µg/ml, respectively, while piperacillin-tazobactam has MIC_{50} and MIC_{90} of 5µg/ml and 10µg/ml.

Figure 1: Antimicrobial susceptibility of *P. aeruginosa* strains from clinical specimens (n=260)



Synergy testing for levofloxacin and piperacillin-tazobactam (Le-Pt)

Synergy testing was done for 11 *P. aeruginosa* isolates for Le-Pt combination (Table 1). On performing FIC with combined MICs ranging from 0.5–8µg/ml, synergy was demonstrated in eight (72.7 per cent) isolates (FIC< 0.5) and for three isolates (27.3 per cent), an additive effect was shown by the chequerboard method. Similar results were elicited by time-kill assays at four hours. The best results were achieved at 2x MICs. Lower MICs did not demonstrate synergy at four hours.

Synergy testing for levofloxacin and cefoperazone-sulbactam (Le-Cfs)

In contrast to Le-Pt, Le-Cfs showed synergy in only three isolates (30 per cent) (Table 2). An additive effect was shown in four isolates (40 per cent), indifference occurred with two (20 per cent), and antagonism with one (10 per cent) by the chequerboard technique. Similarly, by time-kill assay, synergy was demonstrated in three (30 per cent) of these isolates (at 2x MIC) and antagonism in one at four hours with combination of drugs. However, differentiation between addition and indifference could not be done, so addition was seen in six isolates (60 per cent). Surprisingly, synergy was best manifested if either of the strains had high MIC value. On the other hand, in cases with low MIC, indifference was observed.

Discussion

P. aeruginosa is a leading cause of nosocomial infections and is responsible for 10 per cent of all hospital-acquired infections.[13,14] Infections caused by *P. aeruginosa* are sometimes severe and life-threatening, and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of emergence of antibiotic resistance during therapy,[15,16] thus resulting in severe adverse outcomes.[17] β-lactams, aminoglycosides, and fluoroquinolones have been the mainstay for the treatment of *P. aeruginosa* infections.[12] However, the intensive use of antimicrobials inevitably leads to the appearance of strains resistant to these drugs. In our study, out of the various groups tested, most (85.2 per cent) *P. aeruginosa* strains were susceptible to antipseudomonal penicillins with inhibitors, followed by antipseudomonal penicillins (70.4 per cent).

Out of a total of 260 *P. aeruginosa* isolates, 27.3 per cent were found to be multidrug resistant and 19.6 per cent were ESBL producers. Other authors have reported MDR in nearly 45 per cent of *P. aeruginosa* isolates and 25 per cent isolates as ESBL producers.[18] Carbapenems are the only treatment option for such isolates. However, the emergence of carbapenem-resistant *P. aeruginosa* strains due the transmission of plasmid mediated metallo-beta-lactamases has become a challenge for clinicians and microbiologists.

Despite the intensive research in many pharmaceutical industries, no novel class of antibiotic, which resolves the problem of antimicrobial resistance, has been introduced into medical practice.[19] Under these circumstances, combination therapy, employing pre-existing antibiotics, seems a plausable alternative approach for the treatment of infections due to multidrug-resistant strains.

The chequerboard titration method and the time-kill curve technique have been the methods most commonly used to determine in-vitro antibiotic interactions.[20–22] Although each method uses different conditions and end points, there is frequent agreement between the results of the two methods.[23]

In our study, the concordance between these two methods was 71.4 per cent. Other authors have reported agreement between 72–81 per cent.[24] The results of this study demonstrate that the combination of piperacillin-tazobactam and levofloxacin achieve in-vitro synergy in 72.7 per cent of *P. aeruginosa* isolates. In contrast, levofloxacin and cefoperazone-sulbactam combination was synergistic in just 30 per cent of the tested isolates. Synergy was surprisingly not apparent when strains were susceptible to the combination drugs, however, synergy was observed when strains were resistant to one or both the agents. The potential of levofloxacin to act synergistically with piperacillin-tazobactam against resistant isolates may prove advantageous when selecting antimicrobial therapy in institutions with high rates of drug resistance among *P. aeruginosa*.

Conclusion

Synergistic combinations of drugs that are a suitable alternative to carbepenems are required because of the necessity to provide effective, first-line drug treatment options. In this study, Le-Pt emerged as an option for the treatment of multidrug-resistant *P. aeruginosa* infections, and as such could be an alternative therapy before treatment using carbapenems.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ETHICS COMMITTEE APPROVAL

Jawaharlal Nehru Medical College Institutional Ethical Committee in January 2009.

Table 1: Comparison of susceptibility profile, MIC, and synergy testing by chequerboard and time-kill synergy methods for levofloxacin and piperacillin-tazobactam combination

Number of strains tested	Strain no.	Levoflaxin		Piperacillin-tazobactam		Chequerboard technique	Time-kill assay
		Kirby Bauer disc diffusion	MIC (µg/ml)	Kirby Bauer disc diffusion	MIC (µg/ml)		
1	31	+	30	+	10	S	S
2	32	+	30	+	10	S	S
3	49	-	120	-	60	S	S
4	50	-	120	-	60	S	S
5	55	-	240	+	10	A	A
6	11	+	30	+	30	S	S
7	39	+	10	+	10	A	A
8	48	-	120	+	10	A	A
9	45	-	240	+	30	S	S
10	61	-	240	+	30	S	S
11	58	-	240	+	10	S	S

Table 2: Comparison of susceptibility profile, MIC, and synergy testing by chequerboard and time-kill synergy methods for levofloxacin and cefoperazone-sulbactam combination

Number of strains tested	Strain no.	Levofloxacin		Cefoperazone-sulbactam		Chequerboard technique	Time-kill assay
		Kirby Bauer disc diffusion	MIC (µg/ml)	Kirby Bauer disc diffusion	MIC (µg/ml)		
1	39	+	10	+	10	I	I
2	45	-	240	+	30	A	A
3	48	-	120	-	10	I	I
4	58	-	240	-	10	A	A
5	34	+	10	-	128	ANT	ANT
6	46	-	240	-	256	S	S
7	49	-	120	-	256	A	A
8	50	-	120	-	256	A	A
9	38	-	240	+	32	S	S
10	40	+	10	+	64	S	S