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Full Length Research Paper

In vitro synergy profile of some antibiotics against mono and multidrug resistant clinical isolates of *Pseudomonas aeruginosa*

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It is becoming highly challenging for scientific community around the world to battle against rapidly increasing deadly superbugs. Because of heavy exposure of antibiotics and inadequate regimen against infectious organisms, antibiotic resistance in these pathogens has created an immense problem worldwide. Combined antibiotic therapy is frequently recommended for the treatment of such problems. This study is focused to establish *in vitro* antibiotic synergy profiles of combinations of β-lactams and aminoglycoside against drug resistant clinical isolates of *Pseudomonas aeruginosa.* Synergy was determined by checkerboard double dilution method. It was recorded that the combination of cephalexin and amoxicillin was most significantly synergistic (*P*<0.0001) followed by streptomycin and ampicillin (*P*=0.0011), hence these two possible treatment options clearly point to the significance of antibiotic synergy.

Key word: Antibiotic, synergism, multidrug resistant *P. aeruginosa* (MDRPA).

INTRODUCTION

Pseudomonas aeruginosa is a most common causal organism of nosocomial infection (Harris et al., 1999). This organism is considered as a great opportunistic pathogen, which causes infections especially prevalent among patients with cystic fibrosis (CF), acute leukaemia, organ transplants and intravenous drug abuse and urinary tract infections (Prinsloo et al., 2008). Because of heavy antibiotics exposure in clinical settings, a rapid increase in multi drug resistance in this pathogen is of worldwide concern (Marilee et al., 2005).

When it comes to the treatment approach of severe infections in hospitalized patients, an early initiative to start the appropriate antimicrobial therapy improves patient´s recovery, whereas inappropriate therapy is one of the most important causes of hospital mortality. However, therapeutic options for multidrug resistant *P. aeruginosa* (MDRPA) infections in critically ill patients are limited (Marilee et al., 2005).

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The MDRPA isolates may involve reduced cell wall permeability, production of chromosomal and plasmid mediated β-lactamases (Livermore, 1989), aminoglycoside-modifying enzymes (Livermore, 1987), and an active multidrug efflux mechanism (Shahid and Malik, 2004).

Synergy has been observed when resistant anti-pseudomonal drugs were combined *in vitro* against MDRPA with successful clinical application reported in two centers (Marilee et al., 2005). Antibiotic synergy cannot be assumed and should be tested for, prior to commencing treatment with a combination regimen (Prinsloo et al., 2008). Thus in this study we assessed the *in vitro* synergy of eight antibiotics against forty eight clinical isolates of *P. aeruginosa.*

MATERIALS AND METHODS

P. aeruginosa isolates

A total of forty eight *P. aeruginosa* clinical isolates and one reference strain (ATCC-27853) were collected from routine laboratory specimens. The clinical isolates were from different

specimens like pus, swab, sputum, blood and wound discharge of patients attending the health centers of Nagpur district, India. Their species identity was confirmed by biochemical tests including glucose utilization, indole, MR (methyl red), VP (Voges-Proskauer), catalase, oxidase, and by growth characteristic on cetrimide agar.

Antimicrobial agents

Antimicrobial agents, namely Amoxicillin, Cefotaxime, Cefaclor, and Streptomycin were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India, Cephalexin and Ceftriaxone sodium were procured from IFPRESS, Mumbai, India and Ampicillin (Roscillin) was procured from Ranbaxy, India. All drugs were dissolved in their respective solvents and diluted in deionized water and filtered through 0.22 µm (millipore) filter. Drug stocks were stored at -20°C.

Susceptibility and FIC testing

In vitro checkerboard studies on the activity of antibiotics alone and in combination were performed in Muller Hinton broth (Himedia Ltd.) in tube dilutions. Two-fold dilutions (0.125-256 µg mL^{-1}) of each drug or drug combination were tested in two rows. One row was inoculated with 200 µL of an overnight broth culture (contains 10^6 organism mL^{-1}) of the test organism and the second row with the control organism (*P. aeruginosa* ATCC-27853). Results were recorded after tubes were incubated at 37°C for 18 h. Minimum inhibitory concentration (MIC) were determined as the lowest concentration of the drugs (alone or in combination) that inhibited growth. The fractional inhibitory concentration index (FICI) is defined as the sum of the MIC of each drug when used in combination divided by the MIC of the drug used alone. Synergistic effect was recorded when FIC indices ≤0.5; partial synergy when FIC >0.5 but <1.0; additive when FIC =1.0; indifferent when FIC >1.0 but <4.0 and antagonistic when FIC ≥4.0 (Cai et al., 2007).

Susceptibility and FICI values obtained for antibiotics are expressed in percentages, which were subjected to a two-tailed probability test incorporating 47 degrees of freedom using Statpac software.

RESULTS

The *in vitro* results of interaction between penicillins, cephalosporins and streptomycin are presented in Table 1. Statistically significant (P<0.05) synergistic effects were recorded with the combinations of cephalexin and amoxicillin (85.42%), streptomycin and ampicillin (75.00%), cefaclor and amoxicillin (56.25%), cefaclor and streptomycin (45.83%), cefotaxime and amoxicillin (45.83%) and cefotaxime and ampicillin (41.67%) while other combinations were either exhibited low synergistic value or were statistically insignificant results (P>0.05). Only two combinations, ceftriaxone and amoxicillin (25.00%) and ceftriaxone and ampicillin (20.83%), presented partially significant synergistic effect. The combination of cefaclor and ampicillin was found to be having highest significant additive effect (43.75%) followed by cefaclor and amoxicillin (27.08%), cefaclor and ampicillin (14.58%) and ceftriaxone and amoxicillin (12.08%). Most significant indifferent effect was observed with the combination of cefaclor and ampicillin (47.92%), followed by cefaclor and streptomycin (31.25%), ceftriaxone and ampicillin (31.25%) and cefaclor and amoxicillin (14.58%). No antagonism was found in this study.

DISCUSSION

The widespread emergence of antibiotic resistance against nosocomial infecting microorganisms is continuously striking. Despite this, understanding of resistance mechanism and antibiotic synergistic approaches will be the powerful tools for the development of new therapeutic strategies. In the present study we observed that against *P. aeruginosa*, combination of two β-lactams, that is, amoxicillin and cephalexin was found most synergistic (P<0.0001) followed by a β-lactam and an aminoglycoside, that is, ampicillin and streptomycin (P=0.0011). The findings of this study state that the multidrug resistant pathogens can be treated by applying various combinations of antibiotics. Similarly Lang et al. (2000) experimented *in vitro* antibiotic susceptibility to screen double and triple antibiotic combinations for bacterial activity against 75 multiresistant *P. aeruginosa* isolated from 44 cystic fibrosis patients. They found that double antibiotic combination comprising of meropenem and high dose tobramycin exhibited highest bactericidal activity against multiresistant strains of *P. aeruginosa*. Studies of Gunics et al. (2000) using the checkerboard method resulted in the finding that synergy against *P. aeruginosa* was shown by the combination of methylene blue and gentamicin. They also reported synergistic activity of promethazine with ampicillin, tetracycline or erythromycin and the combination of methylene blue and erythromycin against *Escherichia coli*.

The finding of this study illustrates the potential value and necessity of closely monitoring multi-drug resistant pathogens and their susceptibility patterns in nosocomial infections, and combination of antibiotics should be encouraged after *in vitro* studies for better results. General recommendations on antibiotic combinations are difficult to formulate, however, in a clinical situation, each isolate must be judged separately. Such studies should be encouraged by researchers and physicians for a correct drug regimen for timely and efficient treatment of the infections. These strategies would also be helpful for minimizing the economic burden and drug's side effects to the society. Therefore, in the different clinical settings, the systematic investigations of antibiotic synergism would play crucial role in controlling multidrug resistance and provide new insights to overcome nosocomial infections.

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Table 1. *In vitro* interaction of amoxicillin, ampicillin and streptomycin with some cephalosporins and aminoglycoside.

Antibiotic tested	% Sensitivity obtained with Antibiotic alone	Antibiotic in combination with Amoxicillin (n=48)					Antibiotic in combination with Ampicillin (n=48)					Antibiotic in combination with Streptomycin (n=48)				
		% SN (*P* value)	% PS (*P* value)	% AD (*P* value)	% IN (*P* value)	% AN	% SN (*P* value)	% PS (*P* value)	% AD (*P* value)	% IN (*P* value)	% AN	% SN (*P* value)	% PS (*P* value)	% AD (*P* value)	% IN (*P* value)	% AN
Cefaclor	2.08	56.25 (<0.0001)	2.08 (1.0000)	27.08 (0.007)	14.58 (0.0306)	0	6.25 (0.3169)	2.08 (1.0000)	43.75 (<0.0001)	47.92 (<0.0001)	0	45.83 (<0.0001)	8.33 (0.1778)	14.58 (0.0306)	31.25 (0.0002)	0
Ceftriaxone	70.83	58.33 (0.4471)	25.00 (0.0006)	12.50 (<0.0001)	4.17 (<0.0001)	0	4.17 (<0.0001)	20.83 (0.0001)	43.75 (0.0764)	31.25 (0.0049)	0	22.92 (0.0003)	8.33 (<0.0001)	12.5 (<0.0001)	56.25 (0.3708)	0
Cephalexin	25.00	85.42 (<0.0001)	12.50 (0.1552)	2.08 (0.0014)	0	0	20.83 (0.6709)	33.33 (0.4509)	20.83 (0.6709)	25.00 (1.0000)	0	25.00 (1.0000)	20.83 (0.6709)	12.50 (0.1552)	41.67 (0.1551)	0
Cefotaxime	18.75	45.83 (0.0168)	33.33 (0.1596)	10.42 (0.2850)	10.42 (0.2850)	0	41.67 (0.0378)	29.17 (0.2969)	16.67 (0.8096)	12.50 (0.4396)	0	35.42 (0.1139)	12.50 (0.4396)	18.75 (1.0000)	33.33 (0.1596)	0
Streptomycin	29.17	2.08 (0.0004)	31.25 (0.8537)	12.5 (0.0703)	54.17 (0.0544)	0	75.00 (0.0011)	16.67 (0.1994)	6.25 (0.0058)	2.08 (0.0004)	0	-	-	-	-	-
Amoxicillin	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampicillin	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SN: Synergy, PS: Partial synergy, AD: Additive, IN: Indifference, AN: Antagonism.

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