REPORT

In vitro synergistic effect of ciprofloxacin with aminoglycosides against multidrug resistant-*Pseudomonas aeruginosa*

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Abstract: *Pseudomonas aeruginosa* is an increasingly prevalent nosocomial human pathogen. Infections with multidrug-resistant (MDR) *P. aeruginosa* are currently a treatment challenge and requires search for better treatment options. *Purpose of study:* To determine *in vitro* synergistic effect of ciprofloxacin in combination with amikacin and gentamicin against MDR *P. aeruginosa* clinical isolates. *Methods:* Antibiotic resistance pattern of 100 identified clinical isolates of *P. aeruginosa* was determined against eight antibiotics by disc diffusion method at Microbiology Laboratory, Holy Family Hospital, Rawalpindi. For 30 selected MDR isolates, minimum inhibitory concentrations (MICs) of amikacin and gentamicin were determined separately by agar diffusion method followed by combined activity of ciprofloxacin with amikacin and gentamicin by checkerboard agar dilution technique. *Results:* Antibiotic resistance pattern of *P. aeruginosa* isolates was; gentamicin and carbenicillin (94%), amikacin and piperacillin (92%), ceftazidime (90%), colistin (87%), ciprofloxacin (79%) and imipenem (72%). MICs against 30 selected MDR isolates ranged from 32 to ≥128µg/ml for amikacin, and ≥128µg/ml for gentamicin. Synergistic effect was observed in 12/30(40%) isolates for AK+CIP and in 05/30 (16.7%) for CN+CIP. *Conclusion:* Ciprofloxacin in combination with amikacin and gentamicin showed synergistic effect and no antagonistic effect against MDR *P. aeruginosa*.

Keywords: Multi-drug resistant, *Pseudomonas aeruginosa*, amikacin, gentamicin, cipropfloxacin, synergistic effect.

INTRODUCTION

P. aeruginosa is an opportunistic pathogen responsible for the nosocomial infections and is an important cause of mortality, particularly among patients with immuno-suppression, malignancy, cystic fibrosis and burns or traumatic wounds (Rosolini and Mantengoli, 2005; Erdem, 1999; Govan, 1998). Epidemiologically, it is ranked as the fourth cause of nosocomial infections in the United States. Overall prevalence reported was approximately 4 per 1000 hospital discharges (Qarah *et al*., 2008).

Anjum and Asif (2010) studied the prevalence and susceptibility profile of *P. aeruginosa* against various antibiotics in Pakistan and found that 99% of the clinical isolates were resistant to six commonly used antibiotics**.** One of the most worrisome characteristics of the organism is its low antibiotic susceptibility which is attributable to a concerted action of multi-drug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes (Cornelis, 2008)**.** Despite improvements in antimicrobial therapy, *P. aeruginosa* still remains one of the most prominent Gram-negative bacterium causing nosocomial infections (Rosolini and Mantengoli, 2005).

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The development of MDR *P. aeruginosa* is currently one of the greatest challenges to the treatment of infections by this organism (Cornelis, 2008). Therefore newer modalities are required for effective management of infections by this organism. Antimicrobial synergism is one of the approaches that can be used for treatment of such infections. Combination therapy is used with the aim of expanding the antimicrobial spectrum, minimizing toxicity, preventing the emergence of resistant mutants during therapy and obtaining synergistic antimicrobial activity (Zavascki *et al*., 2010; El Solh and Alhajhusain, 2009; Eliopoulos and Moellering, 1999). Several studies have demonstrated that certain antibiotic combinations are more effective than single antibiotics in eradicating serious infections and preserving life (Yamada *et al*., 2007; Oie *et al*., 2003; Mayer and Nagy, 1999).

Many antimicrobial combinations have been studied for synergy *in vitro* and *in vivo* against *P. aeruginosa* (Traugott *et al*, 2011; Dawis *et al*., 2003; Fish *et al*., 2002; Gradelski *et al*., 2001; Mayer and Nagy, 1999) and to the best of our knowledge no such study has been reported against local MDR *P. aeruginosa* as antibiotic resistance pattern of bacterial isolates varies with geographical location and hospitals settings.

This *in vitro* study in Pakistan was undertaken to explore

possible synergistic relationship of ciprofloxacin with gentamicin and amikacin on clinical isolates of MDR *P. aeruginosa* from indoor and outdoor patients of Holy Family Hospital, Rawalpindi, Pakistan.

MATERIALS AND METHODS

Bacterial isolates

One hundred isolates of *P. aeruginosa* were obtained from clinical samples of the patients from Holy Family Hospital, Rawalpindi, Pakistan including wound pus, ear swab, urine, sputum, blood and catheter tips and others. Isolates were identified on the basis of colony morphology and biochemical tests including API 10S (Biomeriux, France). *P. aeruginosa* ATCC 27853 was used as a control strain.

Susceptibility testing

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (Oxoid, UK) using Kirby-Bauer disc diffusion method. Antibiotics tested were; gentamicin (CN), amikacin (AK), ciprofloxacin (CIP), ceftazidime (CAZ), imipenem; (IMP), carbenicilin (CAR), piperacillin (PIP) and colistin (CT). Results of isolates were interpreted as sensitive and resistant according to NCCLS 2001 guidelines (NCCLS 2001). On the basis of susceptibility pattern, 30 MDR isolates were selected to study MICs and combined effect of antibiotics.

Determination of MICs and Synergistic effect

MIC values of AK and CN were determined individually against the 30 MDR *P. aeruginosa* isolates by serial agar dilution method. Antibiotics were used in freeze-dried powder form. The concentration ranges of the antibiotics tested were from 0.5 to 128 µg ml^{-1}.

Synergistic effect of gentamicin and amikacin with ciprofloxacin was determined by checkerboard technique using two fold agar dilution method as was used for MIC determination (Fu and Neu, 1976). The concentration ranges for AK and CN were same as used for MIC determination. Concentration of 1µg ml^{-1} of CIP was added to every dilution of AK and CN to study the combined effect of the antibiotics. After visual inspection of Petri plates for growth, synergistic effect was determined by noting the plates with the lowest concentration of antibiotics in each CIP+AK and CIP+CN. Antimicrobial combination was considered synergistic if there was decrease of four-fold dilution of MIC values and declared antagonistic if result in increase in MIC values while if there is no change in MIC values then the combination has no effect (Fu and Neu, 1976).

RESULTS

Among the 100 clinical isolates of *P. aeruginosa*, 33 were from pus, followed by ear swab (31), catheter tips (11), tracheal tips (8), urine (6), environmental samples (5), blood (3) and high vaginal swab (3).

Resistance pattern of isolates against tested antibiotics was; CN (94%), CAR (94%), AK (92%), PIP (92%), CAZ (90%), CT (87%), CIP (79%), and IMP (72%) (figure1). Isolates with intermediate sensitivity were categorized as resistant. MICs of the 30 selected MDR *P. aeruginosa* isolates were as follows: gentamicin, ≥128µg ml^{-1} and amikacin, 22 isolates had ≥128µg ml^{-1} and 08 had ≥32µg ml^{-1} (table 1).

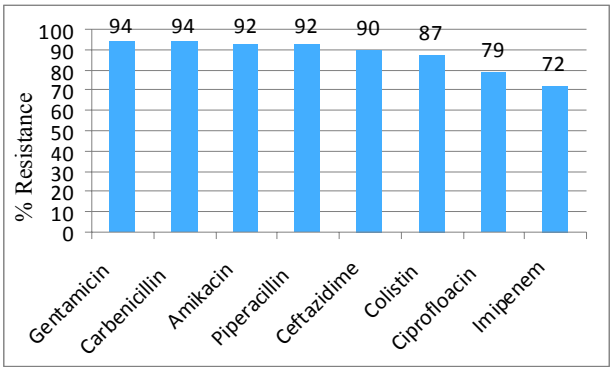

Fig. 1: Antibiotic resistance pattern of clinical isolates of *Pseudomonas aeruginosa*

Synergistic effect was demonstrated by both combinations i.e. CIP+AK and CIP+CN (table 1). Synergism for CIP+AK combination was observed in 12 out of 30 (40%) isolates and for CIP+CN combination in 5 out of 30 (16.7%) isolates.

Table 1: Minimum inhibitory concentrations (MICs) of gentamicin and amikacin alone and in combination with ciprofloxacin against MDR-*Pseudomonas aeruginosa*

Antibiotics	Number of isolates with MIC values (µg ml^{-1})								
	0.5	1	2	4	8	16	32	64	≥128
Gentamicin	-	-	-	-	-	-	-	-	30
Gentamicin + Ciprofloxacin	02	02	-	1	-	-	-	-	25
Amikacin	-	-	-	-	-	-	06	02	22
Amikacin + Ciprofloxacin	05	05	02	-	-	-	02	02	14

DISCUSSION

P. aeruginosa is ranked second among gram-negative bacteria isolated in hospital environment, and a leading cause of nosocomial infections responsible for high morbidity and mortality rate. High prevalence of pseudomonal infections is common among critically ill patients on admission in intensive care unit and those with underlying clinical conditions (Raja and Singh, 2007).

Pseudomonas infections present unique treatment problems because of the extraordinary resistance of these organisms to most antimicrobial drugs especially MDR *P. aeruginosa* isolates (Rossolini and Mantengoli 2005). In present study majority of the isolates were highly resistant towards tested antibiotics and this is in agreement with other studies done by Anjum and Asif (2010) on susceptibility pattern of *Pseudomonas aeruginosa* against various antibiotics.

Aminoglycosides are known frontline antibiotics in the treatment of bacterial infections due to gram-negative bacteria. However, emerging reports showed increased prevalence of resistance against these drugs as observed in this study. In present study, 94% resistance was observed towards gentamicin which is higher than values reported in other studies, with resistance of 40.2% (Fadeyi *et al*., 2005) and 75% (Ogundipeju and Nwobu, 2004). In this study 90% of the isolates showed resistance to ceftazidime, a known antipseudomonal antibiotic.

In vitro testing of fluoroquinolones in combination with many β-lactams or aminoglycosides usually has additive or indifferent effects; occasionally they are synergistic but rarely antagonistic (Chin *et al*., 1986). Synergistic effect of ciprofloxacin with fosfomycin on drug-resistant strains of *P. aeruginosa* was observed by Yamada *et al*. (2007). In present study, MIC values of AK and CN were evaluated for 30 MDR *P. aeruginosa* isolates demonstrated that isolates are highly resistant towards these antibiotics, while when effect of amikacin and gentamicin in combination with ciprofloxacin were studied, it gave promising results.

In this study 40% synergism by AK+CIP and 16.7% synergism by CN+CIP combinations against MDR strains of *P. aeruginosa* is encouraging. The findings are similar to those of certain other investigators who have reported higher *in vitro* synergistic activity of amikacin than of other aminoglycosides (Giamarellou *et al*., 1997). Previous study by Haller (1985) showed that combination therapy with ciprofloxacin and aminoglycoside appears not to implicate any risk of antagonistic drug interactions but did not report any synergistic effects. No antagonistic interactions between ciprofloxacin and aminoglycosides were seen in the present study rather synergistic effects were observed.

CONCLUSION

Combination of ciprofloxacin with amikacin or gentamicin showed synergistic effect and can be a better treatment regimen than the use of gentamicin or amikacin alone against infections caused by MDR *P. aeruginosa* isolates.

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