ORIGINAL RESEARCH PAPER

Pharmaceutical Science

EVALUATION OF POLYMYXCIN B IN COMBINATION WITH CIPROFLOXACIN ANTIBIOTICS AGAINST PSEUDOMONAS AEURUGINOSA

KEY WORDS: Antibiotics, combination therapy, Pseudomonas aeruginosa, disc diffusion method, multi drug resistant, Polymyxin B sulfate, Ciprofloxacin

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ABSTRACT

The fact that bacteria may resist various antimicrobials because they have various antimicrobial-associated genes makes them the primary cause of human infection. Therefore, this study aimed to determine the bacteria resistance to antibiotics. By using the disc diffusion method, antibiotics were tested for their susceptibility to microbes. *Pseudomonas aeruginosa* isolates are completely sensitive to Ciprofloxacin and Polymyxin B sulfate. This study investigated the in vitro activity of the combination of Ciprofloxacin with Polymyxin B sulphate (PMB) at different concentrations against multidrug-resistant (MDR) *Pseudomonas aeruginosa*. The combination effect of Ciprofloxacin and Polymyxin B sulphate antibiotics was evaluated by measuring the zone of inhibition using disc diffusion method. The minimum inhibitory concentration (MIC) of antibiotics was determined using broth microdilution. Our findings suggest that the combination of Ciprofloxacin and Polymyxin B sulphate has a synergistic effect on *Pseudomonas aeruginosa*. As a result, the synergistic combination reported in this study needs to be tested further in vivo before being used in clinical trials.

INTRODUCTION

The rod-shaped, aerobic, gram-negative *Pseudomonas aeruginosa* is a member of the Pseudomonadaceae family. Pseudomonas aeruginosa can colonize and invade a human host to cause dangerous infections [1]. It also adapts readily to the environment it lives in. Infection causing *Pseudomonas aeruginosa* isolates are thought to express a variety of virulence factors. One of the most typical causes of pneumonia is this pathogen. Neutropenia, cystic fibrosis, severe burns, and the installation of foreign devices are risk factors for *Pseudomonas* infections. The majority of people are resistant to infections brought on by *Pseudomonas* species, but these organisms are physiologically very adaptable and can act as opportunistic pathogens in people with compromised immune systems[2]. Life-threatening nosocomial infections like pneumonia, urinary tract infections, and bacteremia, as well as chronic lung infections in people with cystic fibrosis, are all brought on by *Pseudomonas aeruginosa*. Treatment of this infections has become a great challenge due to the ability of his bacterium to resist many of the currently available antibiotics. The World Health Organization (WHO) has recently listed carbapenem resistant *P. aeruginosa* as one of three bacterial species in which there is a critical need for the development of new antibiotics to treat infections. Moreover, excessive use of antibiotics during treatment accelerates development of MDR *P. aeruginosa* strains, leading to the ineffectiveness of the empirical antibiotic therapy against this microorganism [3]. One important gram-negative aerobic bacillus in the differential diagnosis of many illnesses is *Pseudomonas aeruginosa*. This bacterium is significant because it frequently evades the effects of antibiotics and can result in serious hospital acquired infections with a high death rate, particularly in immunocompromised hosts. Many healthcare institutions utilise combination antibiotic therapy for invasive infections with Gram-negative bacteria, especially for certain patient populations, such as those with neutropenia, infections caused by *Pseudomonas aeruginosa*, ventilator-associated pneumonia, and the critically sick. Given that multidrug-resistant Gram-negative pathogens are increasingly causing infections, there is a case to be made for empiric combination treatment. More debatable is whether it is wise to continue combination therapy once an organism has been identified and the antimicrobial susceptibility data have been determined. According to the evidence at hand, rather than taking advantage of in vitro synergy or stopping resistance during definitive treatment, combination antibiotic therapy appears to have the most positive effects on the chance of selecting a successful drug during empiric therapy.

MATERIALS AND METHODS

Bacterial strains:

On regular specimens received at the Microbiology Division, the study was undertaken. *P. aeruginosa* isolates were gathered in December 2022 at MTCC, Chandigarh. It is gathered and transported to the lab for the inoculation of P. aeruginosa bacterial strains (either in sterile bottles or sterile cotton swabs).

Antibiotics:

The following Indian pharmaceutical firms provided the antibiotic powders: Polymyxin-B (POX) was supplied by Sisco Research Laboratories Pvt Ltd in Mumbai. From Research-Lab Fine Chem Industries in Mumbai, Ciprofloxacin (cipro) was acquired. Every powder had a specified potency (g per g powder) that was provided. For each antibiotic, diluents and

solvents were utilized. A wide range of drug concentrations were used to reduce the risk of overlooking potentially useful combinations. In the trials, Ciprofloxacin (2, 4, 6, 8, 10 µg/ml) and Polymyxin B (0.25, 0.5, 1.0, 1.5, 2.0 µg/ml) were introduced at various doses.)[7],[8]

Antibacterial study (Agar diffusion method)

Antibacterial assay was used to determine the growth inhibition of bacteria. Bacteria were maintained at 4°C on broth media before use. Nutrient agar medium was prepared and sterilized at 121°C for 15 minutes. A total of 25ml of nutrient agar was poured into sterile petri dishes and allow settling. Each petri dishes were spread with 0.2ml of different bacterial species *P. aeruginosa*.

An internal diameter 6mm and external diameter 8mm of the cavity was made by using sterile borer, Various extracts are poured into the cavity were made into the set agar containing the bacterial culture. A total of 0.2ml of antibiotic were poured in to the wells with various concentration such as Polymyxin B (0.25, 0.5, 1.0, 2.0 µg /ml), and Ciprofloxacin (2, 4,6, 8,10 µg /ml) used alone and combination. For each bacterial strain controls were maintained were pure solvents instead of antibiotic. The plates were incubated overnight at 37°C. The result was obtained by measuring the zone diameter. [7]

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) were determined at least in duplicate. Broth microdilution (BMD) was used for Polymyxin B and Ciprofloxacin and agar dilution for according to Clinical and Laboratory Standards Institute guidelines [9], with one modification for Polymyxin B for which direct dilution rather than serial dilution was performed to reduce plastic binding [7],[11]. For all other antibiotics, the gradient method (Etest, bioMerieux, Marcy-l'Etoile, France) was applied according to the manufacturer's instructions. Antibiotic susceptibility was classified in accordance with EUCAST clinical breakpoints [10]. Thereafter, various concentration of Polymyxin B antibiotic was added to and then serially dilution. No Polymyxin B products were added to one petri plate, which served as a growth control. After culturing on the Mueller Hinton Agar plates, *P. aeruginosa* was incubated at 37 °C for 18 hours. [10],[11]

Combination of antibiotic:

- Ciprofloxacin 2 µg/ml + Polymyxin B 0.2 ,0.5,1.0,1.5,2.0 µg/ml
- Ciprofloxacin 4 µg/ml + Polymyxin B 0.2 ,0.5,1.0,1.5,2.0 µg/ml
- Ciprofloxacin 6 µg/ml + Polymyxin B 0.2 ,0.5,1.0,1.5,2.0 µg/ml
- Ciprofloxacin 8 µg/ml + Polymyxin B 0.2 ,0.5,1.0,1.5,2.0 µg/ml
- Ciprofloxacin 10 µg/ml + Polymyxin B 0.2 ,0.5,1.0,1.5,2.0 µg/ml

Statistical analysis:

Chi-square was used to compare the in vitro activity of various concentration Polymyxin B products and Ciprofloxacin in P. aeruginosa isolates and was performed triplicate. SPSS version 16 was applied for statistical analysis. Probabilities (p values) less than 0.05 were considered as statistically significant.

RESULT

In the present investigation, the potential interaction between Ciprofloxacin antibacterial activity against *P. aeruginosa* and Polymyxin B sulphate was examined. Results (Table 2) indicated that Polymyxin B sulphate and Ciprofloxacin both had antibacterial efficacy against *P. aeruginosa*. When bacteria were treated with Ciprofloxacin and Polymyxin B sulphate, the zones of inhibition were noticeably bigger than when Ciprofloxacin and Polymyxin B were used separately. Similar outcomes were attained when Ciprofloxacin and Polymyxin B were used, as shown in (Table 3) [12].

Similar result was obtained with the MIC of Ciprofloxacin alone and in combination with Polymyxin B sulphate. Table :1 source the pre-treating bacteria with Polymyxin B sulphate enhance the antibacterial activity of Ciprofloxacin. This is shown by significantly smaller MIC value for the combination at all doses of Polymyxin B sulphate and Ciprofloxacin, as compare to either alone [12] (Table no: 1).

Table No-1: Comparison between the MIC of Ciprofloxacin alone and Ciprofloxacin in the presence of Polymyxin B sulphate against the standard bacterial strain

Standard bacterial strain	MIC (µg/ml)		
	Ciprofloxacin	Polymyxin B sulphate	Ciprofloxacin + Polymyxin B sulphate
Pseudomonas aeruginosa	0.6	0.1	0.07

Table No-2: Zone of inhibition of Ciprofloxacin and Polymyxin B sulphate antibiotic against *Pseudomonas aeruginosa*

Conc. µg/ml	Ciprofloxacin (mm)	Conc µg/ml.	Polymyxin B sulphate (mm)
2	8	0.2	9
4	10	0.5	15
6	14	1	21
8	18	1.5	26
10	22	2.0	31

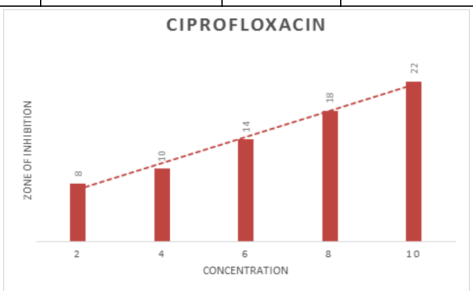

Figure No-1: Zone of inhibition of Ciprofloxacin antibiotic against *Pseudomonas aeruginosa*

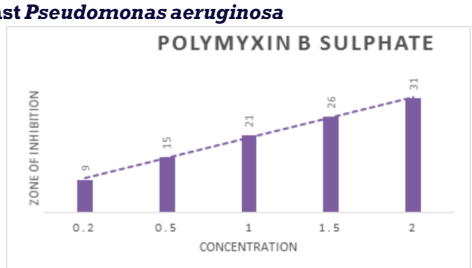

Figure No-2: Zone of inhibition of Polymyxin B sulphate antibiotic against Pseudomonas aeruginosa

Table No-3: Comparison between the zone of inhibition of Ciprofloxacin and Polymyxin B sulphate

Polymyxin B sulphate	Ciprofloxacin mm					
	Conc.	2µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
	0.2 µg/ml	17mm	19mm	20mm	22mm	25mm
	0.5 µg/ml	19mm	20mm	25mm	26mm	28mm
	1.0 µg/ml	23mm	25mm	28mm	29mm	31mm

	1.5 µg/ml	26mm	27mm	31mm	34mm	37mm
	2.0 µg/ml	29mm	35mm	37mm	42mm	46mm

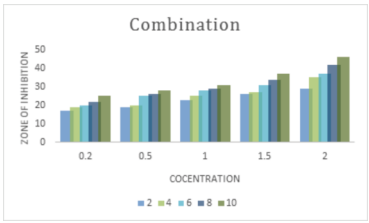

Figure No-3: Comparison between the zone of inhibition of Ciprofloxacin and Polymyxin B sulphate

DISCUSSION

In recent decades, the number of infections caused by antibiotic resistant bacteria has increased rapidly, and hence there is an urgent need for new therapeutic targets and antimicrobials [45]. Moreover, we identified the major bacterial species associated with UTI, pneumonia, wound ear and described the profile of resistance to Ciprofloxacin[14]. Since 2000, the number of bacterial species carrying ESBL genes has increased, and community acquired bacterial isolates with the ability to produce ESBLs that hydrolyse almost all -lactam agents, except for carbapenems, have been reported worldwide. As a result, the clinical use of carbapenems has increased[15]. This in turn caused an increase in the number of clinical bacterial isolates producing -lactamases that have the ability to hydrolyse carbapenems, known as carbapenemases [16]. Thus, the overuse of carbapenems has led to the emergence of carbapenem resistance, which is the ability of bacteria to grow and survive in the presence of clinically relevant carbapenem concentrations [17].

This study investigated the effect of Polymyxin B sulphate and Ciprofloxacin against carbapenemase producing *P. aeruginosa* and reported positive interaction in combination. Of note, enhanced activity was also found with combination of antibiotic to which the strains were highly resistant and in the presence of intrinsic or acquired resistance mechanism. The Polymyxin B sulphate with Ciprofloxacin combination was superior to the single antibiotics against the strains of *P.aeruginosa*. Ciprofloxacin is normally active against both G-Ve and G+Ve (broad spectrum antibiotic). Ciprofloxacin is inability to penetrate the bacterial outer membrane because due to carbapenamase producing by *P.aeruginosa*. However synergistic interaction when used in combination with Polymyxin B sulphate.

The synergistic inhibitory effect observed with combinations of Ciprofloxacin plus Polymyxin B sulphate in nutrient broth (Table 3) may be explained in terms of the Ciprofloxacin modifying cell envelope permeability and thereby facilitating uptake of Ciprofloxacin and Polymyxin. Reported effect of Ciprofloxacin against *P. aeruginosa* appeared to be damage to the peptidoglycan layer of the cell envelope. Various concentrations of Ciprofloxacin had already been shown to enhance bacterial uptake of antibacterial and to enhance antibacterial activity Polymyxin is known to damage cell envelope structures of both stationary and dividing cells (Newton 1954), and would be expected to have a similar enhancing effect on the uptake of Ciprofloxacin [18],[19].

The results presented here support a role for Ciprofloxacin to be used clinically to enhance the antibacterial activity of Polymyxin B sulphate against *P. aeruginosa.*

CONCLUSION

In this study analysis of antibiotic combinations against MDR *P. aeruginosa* revealed favorable interactions. Overall, Polymyxin B combinations with Ciprofloxacin were the most effective against *Pseudomonas aeruginosa* tested in the research. This suggests an additive or synergistic action at 42 hrs.

Because of how it works, Polymyxin B has a lower chance of overcoming the second antibiotic's enzymatic resistance. Through its several resistance mechanisms, *P. aeruginosa* can quickly acquire resistance to medications that would otherwise be quite effective. *P. aeruginosa* that is resistant to treatment is frequently treated with combination therapy. It was also noted that for the therapy to be effective, one susceptible antibiotic was required.

More clinical studies with Ciprofloxacin and Polymyxin B are anticipated to be conducted in the future. Although *in-vitro* studies on MIC, synergy, indicate that some combinations are clearly better to most monotherapies for resistant *P. aeruginosa*, clinical trial results do not support this conclusion, particularly in terms of mortality. Other Gram-negative infections exhibit the same difference. Curiously, the benefits of combinations over monotherapies for bacterial infections like TB are exactly predicted by the *in vitro* benefits of combinations over monotherapies. Because of this, it may be possible that TB and *P. aeruginosa* infections have different underlying causes of death. In cases of TB, the cause of death may be related to a huge bacterial load in the lungs that causes respiratory collapse or direct bacterial infiltration of the heart.

In conclusion, our study shows that combination therapy is a viable and significant therapeutic option for treating *P. aeruginosa* that has developed a high level of resistance.

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