

## Research Paper

# Efflux Inhibition in Clinical Isolates of *Pseudomonas Aeruginosa* Using Sertraline for Ciprofloxacin Resistance Breakdown



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## ABSTRACT

**Background and Aim** The efflux pump in *Pseudomonas aeruginosa* inhibits the effect of ciprofloxacin by releasing quinolones out of the cell. It is important to find compounds to inactivate or inhibit its activity to continue using the antibiotics. The present study was done to investigate using sertraline as an efflux pump inhibitor in *P. aeruginosa* to reduce antibiotic resistance.

**Methods & Materials** *P. aeruginosa* strains were isolated from clinical sources and identified by routine microbiological methods. Resistance of the isolates to ciprofloxacin was evaluated by Kirby-Bauer test. Resistance breakdown was investigated by adding sertraline to the Moller Hinton agar medium and determining the zone of inhibition of ciprofloxacin. Minimum inhibitory concentration (MIC) by microplate dilution method and Minimum bactericidal concentration (MBC) by culture and MTT method were done for the isolates and ATCC 27853. The presence of the efflux pump was evaluated by the phenotypic method using sertraline and serial dilution method of the liquid medium in a microplate, on ciprofloxacin-resistant strains. The presence of the producing gene of this pump was determined by the genotyping method in resistant strains by performing PCR. The standard PAO1 strain of *P. aeruginosa* was used as a positive control.

**Ethical Considerations** This study was approved by the Ethics Committee of the Faculty of Medical Sciences of Islamic Azad University, Brojerd Branch (Code: IR.IAU.B.REC.1401.011).

**Results** Based on Kirby-Bauer test results, three strains were considered resistant to ciprofloxacin. MIC of drug-resistant strains was between 32 and 64 mg/ml and MBC was between 16 and 32 mg/ml. By performing electrophoresis on the PCR products, it was determined that the tested strains contained the *mexA* gene encoding the efflux pump. In the agar medium without sertraline, the zone of inhibition around the ciprofloxacin disc was zero, but after adding sertraline, the diameter of the halo increased to 25 mm. The minimum inhibitory concentration of ciprofloxacin in the isolates before adding 25 µg of sertraline was 128 µg/ml and after adding sertraline, it was 4 µg/ml.

**Conclusion** It was concluded that sertraline inhibited the efficiency of the efflux pump in resistant *P. aeruginosa* isolates and reduced ciprofloxacin resistance.

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## Extended Abstract

### Introduction

**P***seudomonas aeruginosa* plays a key role in various nosocomial infections worldwide. This bacterium is found in many habitats and grows in aerobic conditions.

It causes disease with various virulence factors. Currently, *P. aeruginosa* is inherently resistant to many antibiotics. It might be due to the low permeability of the outer membrane and the efflux pump and the leakage of the enzymes inactivating the antimicrobial agent, making the treatment very difficult. Fluoroquinolones, especially ciprofloxacin, are widely used in the treatment of *P. aeruginosa* infections. The emergence of resistant strains has caused concerns in this regard. The spread of infectious diseases and consequently an increase in drug resistance have been one of the most serious threats to the successful treatment and health of the community. The very high costs of processing an antibiotic and its long time to treat a bacterium have resulted in developing newer ways of using antibiotics in the treatment of bacteria. One way to reduce the bacterial stability is the inhibition of antibiotic disposal systems, such as the efflux pump in bacteria [1]. Sertraline (with trade names of Zoloft and Acentra) is an antidepressant drug from the group of serotonin reuptake inhibitors. It is used to treat depression and is taken orally. Sertraline prevents the reuptake of serotonin by postsynaptic neuroreceptors and an increase in the concentration of serotonin in the central nervous system. Sertraline also slightly inhibits the reuptake of dopamine. Its chemical formula is C<sub>17</sub>H<sub>17</sub>Cl<sub>2</sub>N. Its half-life is between 13 and 45 hours. It has been used in some studies to reduce the function of efflux pump proteins of Gram-negative bacteria, such as *E. coli*.

The present study aimed to investigate using this compound in inhibiting the efflux pump in *P. aeruginosa* [7]

### Materials and Methods

*P. aeruginosa* isolates were collected from clinical centers. Using biochemical reactions and differential diagnosis, *P. aeruginosa* isolates were evaluated in terms of sensitivity to the antibiotic ciprofloxacin from the 12-hour growth of the bacteria based on the CLSI2020 M100 standard guidelines in the form of Kirby-Bauer test by Disk diffusion method with five units of ciprofloxacin disk. The isolates with a zone of less than 18 mm were considered resistant strains. Then, for further examination and verification, MIC was evaluated using the CLSI2020M7-8 standard, and the minimum inhibitory concentration of

ciprofloxacin for the growth of *P. aeruginosa* was determined. The resistant strains in terms of the presence of the efflux pump were identified by PCR method using the primer designed by studying the efflux pump gene.

For resistance breakdown evaluation, 2 % of sertraline was added to the Mueller Hinton agar medium for determining the zone of inhibition of ciprofloxacin.

The microplate dilution method was used for determining the minimum inhibitory concentration. The minimum bactericidal concentration was determined by culturing and MTT methods. Resistance breakdown in broth media was evaluated by the reduction in the minimum inhibitory concentration of the isolates in Muller Hinton Broth and the determination of a significant reduction in bacterial resistance to ciprofloxacin.

### Results

The effect of sertraline on the disc diffusion method was evaluated. In this method, before the addition of sertraline, there was no zone of inhibition (Figure 3), but after the addition of sertraline, the zone of inhibition increased to 25 mm. It means that with the addition of sertraline, the completely drug-resistant bacteria became sensitive.

The results of minimum inhibitory concentration and minimum lethal concentration of ciprofloxacin for drug-resistant strains of *P. aeruginosa* and standard strain *P. aeruginosa* 25873 can be seen in Table 3. The results of the MTT assay were also consistent with the culture results in the case of MBC determination.

The results of the presence of the efflux pump gene showed that the resistant strains had efflux pumps.

The results showed that sertraline can prevent the assembling of the efflux pump in *P. aeruginosa* due to its formulation or interference with its function by forming a complex with the efflux pump proteins. Therefore, fluoroquinolone antibiotics, such as ciprofloxacin, released out of the cell by the efflux pump, prevent the division of the bacteria; thus, the protein-producing function is impaired and the effect of the antibiotic increases.

### Discussion

Based on the results, it was concluded that sertraline could inhibit the efficiency of the efflux pump in resistant *P. aeruginosa* isolates and break the ciprofloxacin resistance in clinical isolates of *P. aeruginosa*.

It is recommended to examine these materials for efflux pumps *in vivo*.

## **Ethical Considerations**

### **Compliance with ethical guidelines**

This research was approved by the Ethics Committee of the Faculty of Medical Sciences of Islamic Azad University, Brojerd Branch (Code: IR.IAU.B.REC.1401.011).

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### **Authors' contributions**

All authors contributed to the research and preparation of this article.

### **Conflicts of interest**

According to the authors, this article has no conflict of interest