



# Current Research in Bacteriology

ISSN 1994-5426

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## **Effective Elimination of Drug Resistance Genes in Pathogenic *Pseudomonas aeruginosa* by Antipsychotic Agent Thioridazine**

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### **ABSTRACT**

*Pseudomonas aeruginosa* is a serious threat in clinical medicine since most isolates are resistant simultaneously to many antibiotics at very high levels. Elimination of these resistances by known pharmacological compounds would be advantageous in successful therapeutic control of various infections caused by this bacterium. Levels of various antibiotics and thioridazine (Tz) resistances were determined in 12 *P. aeruginosa* strains including ATCC 27853 following standard procedure. Elimination or curing of antibiotic resistances was recorded in thioridazine treated strains. Agarose gel electrophoresis was carried out with DNA isolated from both wild type and cured strains following a standard protocol. All 12 strains were multiply resistant to many antibiotics including several  $\beta$ -lactams, cephalosporins, aminoglycosides, fluoroquinolones; however, piperacillin, carbenicillin, amikacin and ciprofloxacin were the only drugs whose resistance levels were much lower. Since, pseudomonas plasmids are fairly large in size, plasmid DNA isolation by normal alkaline lysis process was ineffective. Application of Qiagen kit for isolation of plasmids combined with a manual procedure successfully revealed presence of plasmid bands in wild type strains in the electrophoresis system. Such bands were absent in a few Tz treated cured clones. Piperacillin and carbenicillin are still highly effective against *P. aeruginosa*, while the antipsychotic drug Tz is a potent agent able to eliminate drug resistance plasmids, which are much larger in size than the plasmids of other Gram negative bacteria. Since many antibiotics are administered together to patients suffering from infections caused by *P. aeruginosa*, simultaneous application of thioridazine to such patients may open up a new arena of therapy.

**Key words:** *Pseudomonas aeruginosa*, thioridazine, multi drug resistance, plasmid curing

### **INTRODUCTION**

*P. aeruginosa* is a highly invasive and toxigenic aerobic Gram-negative bacterium. This is non-spore, non-capsulate and usually motile with the help of one or two flagella. The organism readily grows over a wide range of temperature and media. There are several reasons for the preeminence of this microorganism as a human pathogen, like its adaptability, its innate resistance to many antibiotics and disinfectants and its armoury of putative virulence factors (Aendekerk *et al.*, 2005). It is a danger and threat to patients with cystic fibrosis or AIDS and other

immune disorders as well as those requiring long-term hospitalization. *P. aeruginosa* is the most common and lethal pathogen responsible for Urinary Tract Infections (UTI), ventilator associated pneumonia in intubated patients, with directly attributable deaths reaching 38% (Mansouri *et al.*, 2011). Multidrug resistant (MDR) *P. aeruginosa* is found to be resistant to a very large number of antibiotics (Bonomo and Szabo, 2006; Manikandan *et al.*, 2011). Resistance Shahidi-Bonjar *et al.* (2004), Bonjar and Nik (2004) may be due to interplay of various interactions including  $\beta$ -lactamases, mutations, decreased permeability and the activities of efflux pumps (Defez *et al.*, 2004; Abdi-Ali *et al.*, 2007) and presence of drug resistant plasmids (Ranjbar *et al.*, 2007). Although, abundant studies have been carried out to analyse the antibiotic susceptibility pattern of *P. aeruginosa*, there have been a few studies only on the prevalence of MDR (Farrag, 2001; Kohanteb *et al.*, 2007). This may be due to absence of an international consensus regarding the actual definition and concept of MDR in *P. aeruginosa* (Bonomo and Szabo, 2006). Thus, it becomes all the more important that more stress and concentration be provided on this subject so that a solution is available to combat this growing claustrophobic problem of MDR pseudomonad infection. In India, time has come for some serious research and introspection to be made before the MDR *P. aeruginosa* further modifies its genetic make up and turns out to be more deadly than before.

In contrast to an antibiotic, there are medicinal compounds that are used for the therapy of non-infectious pathology and that have distinct antimicrobial properties (Haug *et al.*, 2007). These compounds are termed as 'non-antibiotics'. Non-antibiotics exhibit properties that render them important for the therapy of MDR infections (Dasgupta *et al.*, 2008). Phenothiazines being one of the most important non-antibiotics may be used for determining their anti-pseudomonad potentiality along with their action on elimination of plasmids conferring MDR in *P. aeruginosa*. This study aims to eliminate the drug resistances of *P. aeruginosa* by known pharmacological compounds like phenothiazine, thioridazine that may be a successful therapeutic drug to control various infections caused by this bacterium.

## MATERIALS AND METHODS

**Experimental procedures:** Several strains of *P. aeruginosa* have been obtained from Calcutta Medical College and Hospital, Institute of Postgraduate Medical Education and Research and Bellevue Clinic and Research Kolkata, also from various parts of India and tested for their antibiogram pattern along with *P. aeruginosa* ATCC (American Type Culture Collection), 27853. Purity of each culture of *P. aeruginosa* was checked (Collee *et al.*, 1996).

The antibiotics tetracycline, chloramphenicol, streptomycin, amikacin, penicillin, ampicillin, erythromycin, azithromycin, ofloxacin, ciprofloxacin, ceftazidime, cefotaxime, cefoperazone, ceftriaxone, piperacillin, carbenicillin, imipenem and the non-antibiotics chlorpromazine, thioridazine (Tz), promethazine were obtained in pure dry powder form from their manufacturers in India and stored at 4°C. The tests were also performed with antibiotic discs purchased from Hi-Media, India. The Minimum Inhibitory Concentration (MIC) of an agent is the lowest concentration, which fails to produce any visible growth. The previous concentration showing growth is the level of resistance (Mukherjee *et al.*, 2011).

After determining the level of resistance to Tz in the *P. aeruginosa* strains, plasmid curing test was performed by taking 50% of MIC of Tz with respect to each strain and inoculating the tube with approximately 300 cells. A loop full of growth was plated out on nutrient agar containing Tz so as to produce numerous isolated colonies. At least 100 colonies of a culture were tested for single

or multiple loss of antibiotic resistances at 50% MIC of a particular antibiotic (Dasgupta *et al.*, 2008; CLSI, 2009). Colonies were grown in plates containing Tz at the MIC dose. Then at least 100 colonies of 3 MDR strains, BVC1, BVC2 and BVC3 were stabbed onto plates containing antibiotics at levels just before the MIC dose for each antibiotic respectively. A plain plate containing no antibacterial drug was also stabbed in the same manner; this served as the control. Plates were incubated overnight to detect presence or absence of growth suggested possibility of elimination of plasmid responsible for a certain antibiotic resistance.

Further, colonies in control plate corresponding to no growth in antibiotic plates were inoculated to fresh broth along with original control bacterial strain and kept overnight at 37°C. Next day plasmid DNA was isolated following the method (Birnboim and Doly, 1979; Birnboim, 1983). The Tz treated DNA samples and the wild types were mixed with loading dye and 20 µL of each sample with dye was loaded into agarose gel trough containing agarose gel. Gel was run for 45-60 min and the gel was then stained with ethidium bromide solution, destained with distilled water and placed under BIO-RAD gel docking system for detecting presence or absence of plasmids (Tambekar *et al.*, 2007; Baserisalehi and Bahador, 2008).

## RESULTS

From our studies we observed that the levels of resistances of various strains of *P. aeruginosa* to the mentioned antibiotics were between the range 50-1000 µg mL<sup>-1</sup> in amikacin, 100-1000 µg mL<sup>-1</sup> for piperacillin and carbenicillin, 50-1500 µg mL<sup>-1</sup> in ciprofloxacin and norfloxacin, 500-1000 µg mL<sup>-1</sup> with respect to imipenem, 30-2000 µg mL<sup>-1</sup> for azythromycin, 500-3000 µg mL<sup>-1</sup> in tetracycline and streptomycin, 1000-3000 µg mL<sup>-1</sup> for chloramphenicol, 5000 µg mL<sup>-1</sup> in respect of ampicillin and erythromycin, 500-1000 µg mL<sup>-1</sup> for ceftazidime, ceftriaxone, cefoperazone and 2000 µg mL<sup>-1</sup> for Tz as shown in Table 1.

Thus, the strains when tested against Tz and the antibiotics mentioned above they were found to be resistant to almost all of them at very high levels. Piperacillin, carbenicillin, amikacin and ciprofloxacin were only antibiotics that could inhibit these organisms at rather low levels. When these strains were treated with 50% level of MIC of Tz in liquid culture followed by inoculation on

Table 1: Resistance pattern of *P. aeruginosa* with respect to antibiotics and thioridazine

<i>P. aeruginosa</i>	Antibiotic (µg mL <sup>-1</sup> )												Th (µg mL <sup>-1</sup> )
	Pc	Cb	Pp	Im	Cz	Ct	Cx	Sm	Ak	Tc	Cm	Cr	
ATCC 27853	2000	200	100	500	250	500	1000	2000	200	1500	2000	100	2000
C15	>3000	500	200	1000	500	500	1000	2000	500	1500	2000	100	2000
C17	>3000	500	200	1000	500	500	1000	2000	500	1500	2000	100	2000
APC1	>3000	500	500	>1000	1000	500	>1000	>2000	500	2000	2000	100	2000
732	>3000	>1000	500	>1000	1000	500	>1000	>2000	1000	2000	2000	100	2000
AMRI 100	>3000	1000	>1000	>1000	>1000	>1000	>1000	>2000	>1000	>2000	>3000	>1000	>3000
BVC1	>3000	200	100	>1000	250	1000	>1000	2000	500	2000	2000	100	2000
BVC2	>3000	>1000	500	>1000	>1000	1000	>1000	>2000	500	2000	2000	100	2000
BVC3	>3000	1000	>1000	>1000	>1000	>1000	>1000	>2000	>1000	>2000	>3000	>1000	>3000
BVC4	>3000	1000	500	>1000	500	1000	>1000	2000	500	2000	2000	100	2000
BVC5	>3000	>1000	>1000	>1000	>1000	>1000	>1000	>2000	>1000	>2000	>3000	>1000	>3000
7	>3000	500	500	1000	500	500	1000	2000	500	2000	2000	100	2000

Pc: Penicillin, Cb: Carbenicillin, Pp: Piperacillin, Im: Imipenem, Cz: Ceftazidime, Ct: Ceftriaxone, Cx: Cefoperazone, Sm: Streptomycin, Ak: Amikacin, Tc: Tetracycline, Cm: Chloramphenicol, Cr: Ciprofloxacin, Tz: Thioridazine

solid media many of the strains were found to lose several of their antibiotic resistances (Fig. 1). This was confirmed by elimination of plasmids with the help of DNA isolation and gel electrophoresis technique (Fig. 2).

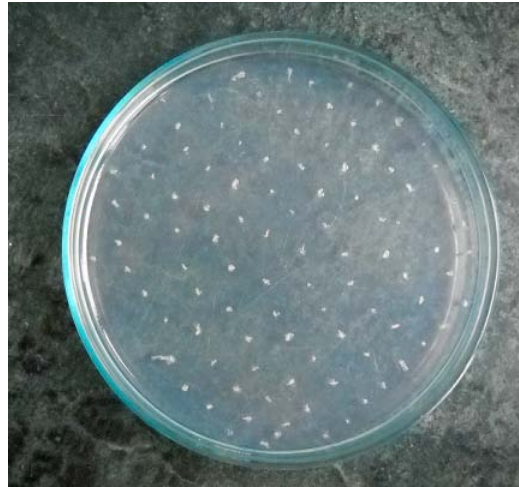


Fig. 1: Tz treated *Pseudomonas* colonies stabbed on to antibiotic containing plates (less than MIC value)

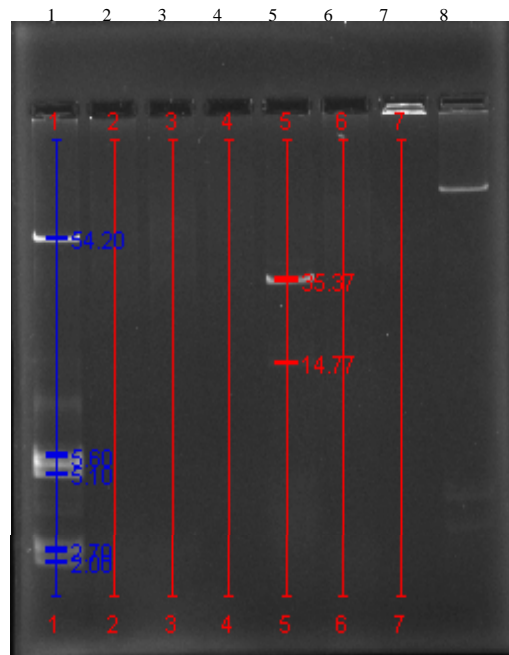


Fig. 2: Agarose gel electrophoresis, Lane 1: *E. coli* K 12 (V517) marker, Lane 5: Wild type *Ps. BVC2*, Lane 6 and 7: Tz treated strain with plasmid missing. Lane 8: Standard *Shigella flexneri* (YSH6000) plasmid

## DISCUSSION

This study suggests the action of Tz in eliminating antimicrobial resistance in 3 MDR *P. aeruginosa* strains and rendering them sensitive to antibiotics at lower levels (Molnar and Schneider, 1978; Molnar *et al.*, 1980, 1992, 2004).

Thus, simultaneous application of Tz would not only act as an additional antibacterial agent but also would help to eliminate the drug resistant plasmids from the infectious bacterial cells (Spengler *et al.*, 2006). Therefore, the patients suffering from MDR *Pseudomonas* infections may be administered Tz at standard human doses along with antibiotics.

The present study would help to estimate the prevalence of infections due to MDR *P. aeruginosa*, often resulting in mortality, increased hospital costs and prolonged stay. The clinical and economic impact of MDR *P. aeruginosa* is substantial and greatly worrisome. An international agreement on the definitions and a simultaneous secondary pathway in treatment of such a bacterium could potentially facilitate an orchestrated response against this pathogen.

## CONCLUSION

MDR *P. aeruginosa* infection is very often life threatening to patients with compromised immunity and its increasingly growing resistance to various antibiotics are creating massive problems in such infections. Alternative, therapy of treatment with drugs other than antibiotics should be searched for to control and combat the fatal consequences of this infection.

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