

# Resistance to Antibiotics and Heavy Metals of *Pseudomonas Aeruginosa* Isolated from Natural Waters

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## ABSTRACT

Heavy metal resistance of *Pseudomonas aeruginosa* may be associated with plasmids which are also responsible for antibiotic resistance. The concentration of heavy metal pollutant in the environment is relatively high due to the toxic substance liberated from the industrial effluents. The high occurrence of antibiotic-resistant bacteria in natural water could be related to the widespread use of antibiotic, with possible public health hazard. The foremost aim of this present work is find out, resistance to antibiotics and heavy metals of *P.aeruginosa* isolated from natural water.

**Keywords :** P. Aeruginosa, Heavy Metals, Antibiotics

## I. INTRODUCTION

Microorganisms that are resistant to both antibiotics and heavy metals have been frequently isolated from different aquatic environments (30). The high occurrence of antibiotic-resistant bacteria in natural water could be related to the widespread use of antibiotics, with possible public health hazard (29). The detection of *Pseudomonas aeruginosa* in an aquatic natural environment is generally related to pollution come from sewage (10,16,19,22). An important characteristic of this microorganism is its high resistance to antibiotics, heavy metals and other environmental factors which can be related to its survival capability in aquatic environment (8,13,21,25). The presence of a large number of resistant bacteria in natural habitats can pose a public health risk (29). *Pseudomonas aeruginosa* is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. It is physiologically versatile and flourishes as a saprophyte in warm moist situations in the human environment, including sinks, drains, respirators humidifiers and disinfectant solutions. It causes urinary tract infection, respiratory system infection, dermatitis, soft tissue infection, bacteremia bone, gastro intestinal infection and immuno suppressed (5). The present investigation was, therefore to determine the isolation of antibiotic and heavy metal resistance bacteria *Pseudomonas aeruginosa* from natural water.

## II. METHODS AND MATERIAL

### Sample Collection

Water sample were collected from different sites in the Chennai area and the samples from different sources namely portable water, sewage water, sea water were collected for the analysis.

### Processing

The collected samples were immediately transferred to the laboratory for investigation. Color and turbidity were observed.

### Bacteriological Examination

*Pseudomonas aeruginosa* were determined using the membrane filtration technique on an yeast extract medium and incubated for 24h. All colonies were recovered on yeast extract agar and the strains were purified on tryptose soya agar. The colonies isolates were primarily identified on the basis of their colonial morphology, pigment production and on oxidase test. Conformation of identity was based on the standard biochemical methods (17,26).

### Antibiotic Susceptibility

The susceptibility of *P.aeruginosa* isolates to various antibiotics was determined by the standard disc-agar

diffusion method on Mueller Hinton agar (18). The following antibiotic discs were used Ampicillin (AM) 10µg, Amikacin (AK) 30µg, Streptomycin (S) 10µg, Tetracyclin (TE) 30µg, Carbinicillin (CB) 10µg, Gentamicin (GM) 10µg.

### Examination of Heavy Metal Resistance

The resistance to heavy metals was evaluated by the agar dilution method (31). The strains of *P.aeruginosa* were assayed against the following concentrations of different metal salts Zinc sulphate 1600µg/ml, Mercuric chloride 10µg/ml, silver nitrate 128µg/ml. Appropriate stock solutions of different metallic salts were prepared in distilled water, sterilized by membrane filtration (0.47µm) and kept at 37°C for 24h.

## III. RESULTS AND DISCUSSION

### Resistance and sensitivity patterns of *Pseudomonas aeruginosa*:

Antimicrobial chemicals are also known as chemotherapeutic agents the sensitivity of micro-

organism to various chemotherapeutic agent varies antibiotic sensitivity test is performed on the basis of agar diffusion method and the results were tabulated. Table 1 shows that, *P.aeruginosa* isolated from portable water, sea water and sewage water were resistant to ampicillin, tetracyclin and gentamicin. On the other hand *P.aeruginosa* were sensitive to amikacin and streptomycin. The given antimicrobial resistance patterns were similar to those reported by Al-Jebouri 1985. The wide spread use of antibiotics in medicine and other disciplines is accepted as a major factor for the increase in the incidence of antibiotic resistance especially among gram negative bacteria. Antibiotic resistant strains of *P.aeruginosa* causing diseases such as otitis, meningitis, urinary tract, ear and eye infections and skin rashes have been previously isolated from clinical materials by Shohayeb et al., 1999. The present study shows that, the coliform bacilli are generally considered to be indicators of the bacteriological quality of water supplies. The frequency of antibiotic resistance among coliform bacteria varied from sample to sample and this is probably dependent upon the source of contamination of the water.

**Table 1.** Antibiotic Sensitivity of *Pseudomonas Aeruginosa*

Antibiotic disc	Zone of inhibition (mm)			Resistance / Sensitive		
	Strains of portable water	Strains of sea water	Strains of sewage water	Strains of portable water	Strains of sea water	Strains of sewage water
Ampicillin	No-zone formation	No-zone formation	No-zone formation	R	R	R
Amikacin	18mm	17mm	19mm	S	S	S
Streptomycin	18mm	19mm	17mm	S	S	S
Tetracyclin	No-zone formation	No-zone formation	No-zone formation	R	R	R
Carbinicillin	No-zone formation	No-zone formation	No-zone formation	R	R	R
Gentamicin	10mm	13mm	12mm	R	R	R

## Heavy Metal Resistance

Table 2 shows that, *P.aeruginosa* strains to various concentrations of the three heavy metal ions were investigated, and the minimum inhibitory concentration is defined as the lowest concentration of metals which completely inhibits the growth of organisms. The present study reveals *P.aeruginosa* strains of portable water, sea water and sewage water were resistant to Hg, Zn and Ag. Which were similar to thr resistant obtained to those reported by Joly et al.,1976. The strains isolated from sea water shows the higher frequency of resistance which have been correlated with the report of De Vicente 1990, although they were lower than the frequencies observed for strains of *P.aeruginosa* isolated from different sources (8,30,31). The resistance to a particular heavy metal has already been reported to correlate with antibiotics and other heavy metals resistance in certain organisms (7,29,30) and the role of plasmids in conferring resistance to antibiotics and metals has also previously demonstrated (20,30,31). The study of heavy metals sensitivity shows that every strain in resistant to most of the heavy metal to some limit.pressure from heavy metal salts naturally present in water may favour persistence of metls – resistant bacteria and on different microbes and pressure of micro environment and the microbes contain transferable factor in certain micro environments may provide conditions where resistance transferred could occur.

**Table 2.** Heavy Metal Resistance

Heavy metal	Concentration of heavy metal	Minimum inhibitory concentration (µg)			Resistance / Sensitivity
		Clea	Clea	Clea	
Ag 128µg/ ml	64	Clea	Clea	Clea	Resistant
	32	r	r	r	
	16	Clea	Clea	Clea	
	8	r	r	r	
	4	Clea	Clea	Clea	
		Turbid	Turbid	Turbid	
		Turbid	Turbid	Turbid	
Hg 10µg/m	5.0	Clea	Clea	Clea	
	2.5	r	r	r	

1	1.2	Turbid	Turbid	Turbid	Resistant
	0.6	id	id	id	
	0.3	Turbid	Turbid	Turbid	
		id	id	id	
Zn 1600µg/ ml	800	Clea	Clea	Clea	Resistant
	400	r	r	r	
	200	Clea	Clea	Clea	
	100	r	r	r	
	50	Clea	Turbid	Turbid	
		Clea	Turbid	Turbid	
		r	id	id	
		Turbid	Turbid	Turbid	
		id	id	id	

## IV. CONCLUSION

From the results, it is clear that *P.aeruginosa* is highly resistance to antibiotics and heavy metals. So, it is very difficult to eliminate the organisms from the environment. Proper sanitation measure can full fill avoiding *P.aeruginosa* to some extent. The results reported in this paper provide evidence that polluted water may play important role as reservoirs of antibiotic and heavy metal resistance and enhanced as a potential public health hazard.

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