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Isolation of Pseudomonas Species from Waste Water in Aurangabad, as Reservoir of Antibiotic Resistant Determinants

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Abstract

Pseudomonas species are opportunistic pathogens causes wide range of diseases including Cystic fibrosis, Sickle cell aneamia etc. Because of multiple drug resistance (MDR) Pseudomonas species are threat to public health. The result shows occurrence of Pseudomonas species in waste water are as follows. Pseudomonas fluorescence (10%), Pseudomonas aeruginosa (14%) and Pseudomonas putida (20%) respectively. Disc diffusion assay of Pseudomonas isolates showed approximately 100% resistance to Penicillin, Tetracyclin, Ampicillin and susceptible to Ciprofloxacin, Gentamycin.

Keywords: Pseudomonas, Multidrug resistance (MDR).

Introduction

Antibiotic resistance in bacteria has been recognized as a major medical problem to the mankind. Therefore it is necessary to study their antibiotic resistance and susceptibility[1]. Most of the studies have been focused on enteric pathogens such as E. Coli, Aeromonas, Compylobacter[2,4,5]. However environment is the reservoir of antibiotic determinants, and spread antibiotic resistant bacteria in human beings through plasmid, transposons [2]. Waste water treatment plants have been reported as important reservoirs of antibiotic resistant organisms which could persist in the treated effluent and subsequently released into the natural environment [8–10] and thus impact on the ecology of antimicrobial resistance in bacterial populations [11–13]. However, reports of commensal bacteria including the pseudomonas as sources of antibiotic resistance determinants in the environment are rare.

Pseudomonas species are Gram negative motile rods belonging to the family pseudomonaceae and found in various environments. Their ability to utilize different organic compounds as carbon and energy source as well as survival in the apparent absence of nutrients has been attributed to their genetic versatility which translates into enhanced metabolic activity with exceptional ability to adapt and colonize a wide variety of ecological niches including water, soil and rhizosphere [14]. Pseudomonas spp. are so well adapted in their

environment that they survive in extremes which includes temperatures ranging from 4°C to 43°C, and weak ion concentrations, among others. In this study, we assessed the incidence of Pseudomonas spp. in wastewater and their antibiotic resistance.

Materials and method

Sample was collected from wastewater treatment plant of Aurangabad and serially diluted in sterile distill water upto 10-6. 100µL sample from last three dilutions was plated on Pseudomonas isolation agar purchased from Himedia and incubated overnight at 37°C. On the basis of Morphological, Cultural and Biochemichal characteristics the isolates were identified by Bergey's Manual of Determinative bacteriology as a Pseudomonas aeroginosa, Pseudomonas fluorescence and Pseudomonas putida.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the BSAC Disc Diffusion Method [17,32] with Nutrient agar as the growth medium. Antibiotics were selected to represent some major classes of antibiotic and anti-pseudomonal antibiotics used as first line drug for pseudomonal infections. Antibiotics used in the study include penicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), chloramphenicol (30µg), tetracycline (10µg), cefotaxime (30µg), ofloxacin (5µg), ampicillin (25 µg). Antibiotic discs were purchased from Himedia

Results

Isolation and identification of isolates

Gram's staining of the colonies were performed and selected colonies used for biochemical reactions such as catalase test, oxidase test, gelatin liquefaction test, H2S production and IMViC test. Colonies were identified by referring Bergey's Manual of Determinative bacteriology as a Pseudomonas aeroginosa, Pseudomonas fluorescence and Pseudomonas putida.

Antibiotic susceptibility test

The antibiogram of Pseudomonas species is shown in table 1 and graph 1. All the isolates were identified as 100% susceptible to ciprofloxacin and gentamicin. Conversely, all the isolates were found variably resistant to penicillin, ampicillin, tetracycline and other antibiotics.

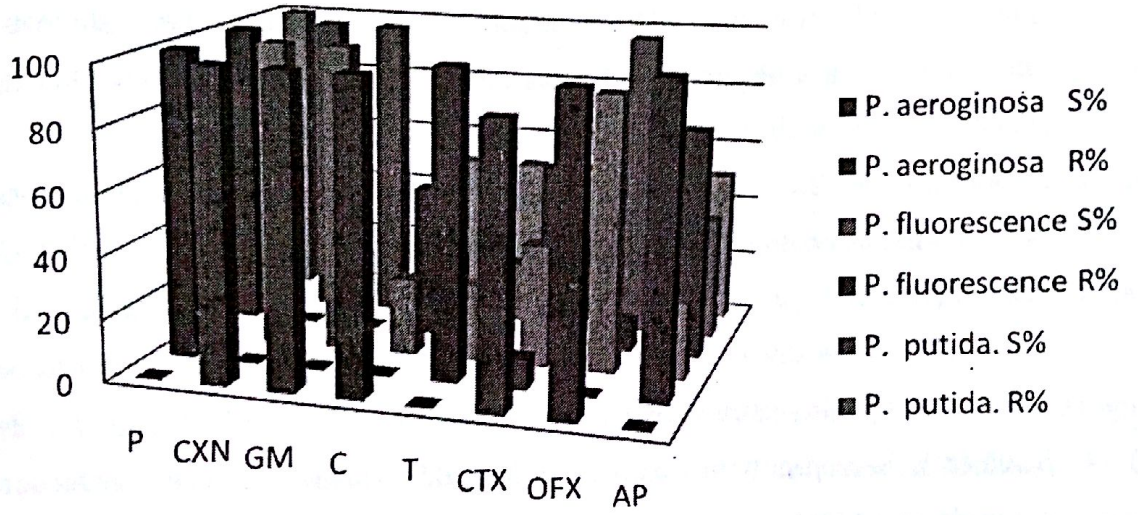
Table 1: Antibiotic resistance of Pseudomonas species isolated from waste water in Aurangabad

Antibiotic	Pseudomonas aeroginosa		Pseudomonas fluorescence		Pseudomonas putida	
	S%	R%	S%	R%	S%	R%
Penicillin	0	100	0	100	0	100
Ciprofloxacin	100	0	100	0	100	0
Gentamycin	100	0	100	0	100	0
Chloramphenicol	100	0	25	50	20	50
Tetracyclin	0	100	25	50	20	50

Antibiotic	Pseudomonas aeroginosa		Pseudomonas fluorescence		Pseudomonas putida	
	S%	R%	S%	R%	S%	R%
Cefotaxime	90	10	40	60	30	50
Ofloxacin	100	0	90	10	100	0
Ampicillin	0	100	25	75	40	50

(S%= Susceptibility), (R%= Resistance)

Antibiotic resistance 0f *Pseudomonas* species from waste water in Aurangabad



Legend : P= Penicillin, CXN= Ciprofloxacin, GM= Gentamicin, C= Chloramphenicol, T= Tetracyclin, CTX= Cefotaxime,OFX= Ofloxacin, AP= Ampicilin. (S%= Susceptibility), (R%= Resistance)

Conclusion

Resistance to different classes of antibiotics shown by the Pseudomonas species isolated from waste water is an indication of the potential of the environment as a reservoir for antibiotic resistant organisms. Wastewater treatment process has been put forward as a potential vehicle for the selective enhancement and increase of multidrug resistant bacteria in the aquatic environment. The antibiotics like ciprofloxacin and gentamicin are the only broad spectrum antibiotics that showed high activities against all the isolates, while other antibiotics showed little or no activity against them, thus suggesting these isolates to be multi-drug resistant and pose a threat to public health. The presence of antibiotic resistance in these environmental isolates suggests Pseudomonas species act as a carriers and sources of antibiotics resistant.

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