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Studies on antibiotic resistance of *Pseudomonas aeruginosa* isolated from various sample with special reference to the antibacterial activity of its pyocyanin pigment

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ABSTRACT

Pseudomonas aeruginosa is a bacterium responsible for causing life threatening infections like nosocomical infections, life threatening infections in immune compromised persons. The bacterium's virulence depends on the numerical abundance of cells associated with extracellular factors. The study was taken up to isolate and to understand the biochemical characterization of *P. aeruginosa* isolated from pus sample (S1), urine samples (S2) Sputum sample (S3) and soil sample (S4). The multiple drug resistance property of *P. aeruginosa* was clearly demonstrated by Kirby-Bauer's disc diffusion method. *Pseudomonas aeruginosa* (S1) isolated from pus sample was selected for pigment studies, since this strain showed good resistance to various antibiotics than others. Pyocyanin (1-hydroxy-N-Methyl phenazine) is a bluish green, chloroform soluble, cytotoxic pigment secreted by the bacterial species of *P. aeruginosa.* Tech agar was used for the production of pyocyanin pigment and the pigmented broth tube was centrifuged and the pigment was extracted using choloroform. The extracted pigment showed antibacterial activity against *E.coli, Proteus vulgaris* and *Staphylococcus aureus*.

Key words: Clinical samples, *Pseudomonas aeruginosa,* Pyocyanin, and Antibacterial activity.

INTRODUCTION

The members of Pseudomonas are strictly aerobic, catalase positive, oxidase positive, Gram negative motile bacilli. Their metabolism is respiratory but never fermentative. An RNA group 1 comprises the fluorescens group including *P. aeruginosa, P. fluorescens and P. putida*[1]. *Pseudomonas aeruginosa* can occur as 5 distinct types ranging from dwarf colonies to a large mucoid type[2]. Three types of bacteriocins (pyocins) are produced by *P. aeruginosa* which are known as R, F and S[3].

Pseudomonas aeruginosa produces a variety of enzymes and toxins[4]. Besides this, remarkable number of bacterial factors have been postulated playing a role in *P. aeruginosa* infections[5]. Further, *P. aeruginosa* secretes a number of virulence factors, including elastases, rhamnolipids, alginate, exotoxin A, exoenzyme S, and phospholipase C, whose virulent properties have been elucidated well[6, 7]. Two *in vivo* murine respiratory tract infection models, acute and chronic, have been demonstrated as the absolute necessity of pyocyanin biosynthesis for *P. aeruginosa* virulence [8].

Pseudomonas aeruginosa is a bacterium responsible for severe nosocomial infections, life-threatening infections in immuno- compromised persons. The bacterium's virulence depends on a large number of cells-associated with extracellular factors[9]. Chronic *Pseudomonas aeruginosa* infections occur frequently with the

immunocompromised, the aged, and those with bronchiectasis[10], and otitis externa[11].

Pyocyanin (1-hydroxy-N-methylphenazine) is a cytotoxic pigment secreted by the bacterial species *Pseudomonas aeruginosa,* which frequently infects the lungs of immunosuppressed patients. Pyocyanin toxicity results presumably from the ability of the compound to undergo reduction by NAD (P)H and subsequent generation of superoxide and H_2O_2 directly in the lungs[12]. Pyocyanin triggers tissue damage mainly by its redox cycling and induction of reactive oxygen species[13]. Anti – *Staphylococcal* activity by *Pseudomonas aeruginosa* was investigated through the use of the reverse agar plate and the filter paper stamp methods[14].

MATERIAL AND METHODS

Sample collection and Isolation

Different types of clinical samples such as pus samples, urine samples, and sputum samples were collected from hospitalized patients at Meenakshi Mission Hospital and Research centre, Madurai. *Pseudomonas aeruginosa* was isolated from soil by serial dilution technique the samples were streaked on blood agar and Macconkey agar. The selected isolates were characterized by different biochemical tests. Antibiotic sensitivity test was done by Kirby-Bauer's disc diffusion method.

Production of pigment and extration

Pseudomonas-Agar P promotes pyocyanin production[15]. Pseudomonas-Agar P was prepared for the production of pyocyanin. The plates inoculated with isolated strains and incubated at 35 $\pm$ 2°C for 24 hours. If the isolate fails to grow or grow slowly, the isolate may be re incubate at 30°C for 1-2 days and observe for growth and pigment production. The presence of pyocyanin may be confirmed by adding several drops of chloroform and observe for a blue colour in the chloroform [16].

Extraction of non polar compounds from crude *P. aeruginosa* culture supernatants was performed by adsorption to an octadecylsilance bonded phase chromatographic material. After thorough washing of sep-pak cartridges with Sorenson buffer, pH 7.6, 20ml of bacterial culture supernatants diluted 1:1 in buffer were passed through the cartridges. After additional washing with buffer, sequential elution with 5ml of chloroform and 5ml of methanol were performed[17].

RESULTS AND DISCUSSION

Psedomonas aeruginosa was isolated from pus samples, urine samples, sputum samples and soil sample. S1-isolate from pus sample; S2-isolate from urine sample; S3-isolate from sputum sample and S4-isolate from soil sample were selected. They were confirmed by morphological, bacteriological and biochemical characterization (Table 1). *Pseudomonas aeruginosa* colonies have a spreading habit, and give a characteristic grape like fruity odour due to the production of aminoacetophenone. Bio chemical reaction on TSI show alkali / alkali (slant / butt)-typical of non fermenters, H_2S negative[18].

The multiple drug resistance property of *pseudomonas aeruginosa* was clearly demonstrated by Kirby-Bauer's disc diffusion

Table 1: Biochemical characterization of *Pseudomonas aeruginosa*

Biochemical test	Pathogenic strains from clinical sample			Strain from soil sample
	S1	S2	S3	S4
Indole	-	-	-	-
TSI agar	-	-	-	-
Citrate	+	+	+	+
Urease	+	+	+	+
Mannitol fermentation	-	-	-	-
Mannitol motility	+	+	+	+
Catalase	+	+	+	+
Oxidase	+	+	+	+

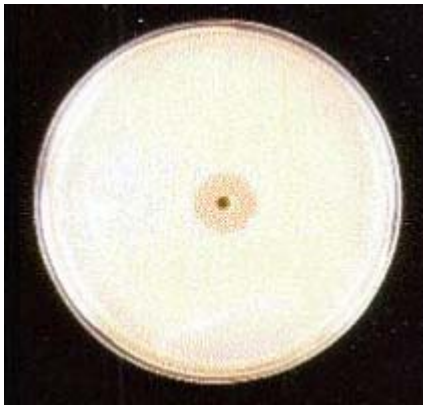

Fig. 1: Study of antibacterial activity of pyocyanin against *E. coli*

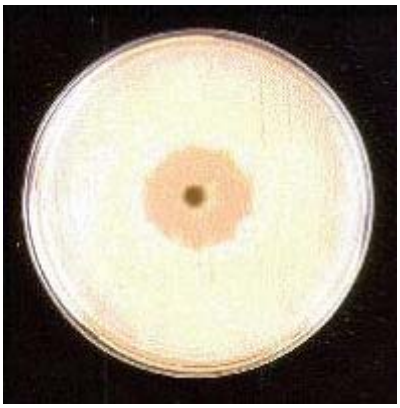

Fig. 2: Study of antibacterial activity of pyocyanin against *Proteues*

Fig. 3: Study of antibacterial activity of pyocyanin against *Staphylococcus aureus*

method. Both the clinical and soil isolates showed antibiotics resistance to ampicillin, cefazolin, cofactor, cefdinir and Nolidixic acid. But *P. aeruginosa* sensitive to amikacin, cefatazidime, gentamycin and cefoperazone as evident by the inhibition zone, prove that the drug will be clinically effective (Table 2). Pseudomonas agar plates were used for production of pyocyanin pigment. The antibacterial activity of pyocyanin pigment of *P.aeruginosa* was analysed. The extracted pigment showed antibacterial activity against *Escherichia coli, Proteus vulgaris, Staphylococcus aureus.* (Table 3, Fig. 1 3). Pyocyanin is a cytotoxic pigment secreted by the bacterial species *P. aeruginosa.* Approximately 50% of clinical *Pseudomonas aeruginosa* isolates were found to produce pyocyanin at 37°C. Finally conclude that the pigment of *Pseudomonas aeruginosa* showed good antibacterial activity against selected bacterial culture. Among this, *Proteus vulgaris* strain highly sensitive to pyocyanin.

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Table 2: Antibiotic susceptibility test (Disc Diffusion Method)

S. No.	Antibiotics	Strain isolated from pus (S1)		Strain isolated from urine (S2)		Strain isolated from Sputum (S3)		Strain isolated from Soil (S4)	
		Zone diameter (mm)	Sensi-tivity	Zone diameter (mm)	Sensi-tivity	Zone Diameter (mm)	Sensi-tivity	Zone diameter (mm)	Sensi-tivity
1	Ampicillin	-	R	-	R	-	R	-	R
2	Amoxycillin + Clavulanic acid	-	R	14mm	S	-	R	-	R
3	Amikacin	14mm	S	20mm	S	23mm	S	23mm	S
4	Cefdinir	-	R	-	R	-	R	-	R
5	Cefazolin	-	R	-	R	-	R	8mm	R
6	Ceftazidime	20mm	S	10mm	R	20mm	S	35mm	S
7	Co-Trimoxazole	-	R	-	R	10mm	R	-	R
8	Cefaclor	-	R	-	R	-	R	-	R
9	Gentamicin	-	R	21mm	S	17mm	S	18mm	S
10	Ceficime	-	R	9mm	R	8mm	R	13mm	S
11	Nalidixic acid	-	R	-	R	7mm	R	-	R
12	Cefoperazone	20mm	S	13mm	S	20mm	S	29mm	S

(-) No zone, (R) Resistant, (S) Sensitive

Table 3: Antibacterial activity of pyocyanin pigment against few bacterial strains

Pigments	Diameter of zone of inhibition in mm		
	Escherichia coli	***Proteus vulgaris***	***Staphylococcus aureus***
Pyocyanin	14	19	15

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