Antibiogram profile of isolated *Pseudomonas spp*

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Abstract: ***The study conducted to isolate Pseudomonas spp. from soil and water sources. The identification of Pseudomonas spp. performed by staining and biochemical testing. The biochemical test suggests isolated strains from garden soil, petroleum-contaminated soil, edible oil-contaminated soil, metal contaminated soil, and contaminated seawater, possess similar characteristics like Pseudomonas fluorescence, Pseudomonas aeruginosa, Pseudomonas alkaligens, Pseudomonas mendocina, Pseudomonas putida Respectively. Molecular fingerprinting is necessary to confirm their presence. Antimicrobial susceptibility of Pseudomonas spp. against different antibiotics was performed by Kirby-Baur Method. In the susceptibility assay, five different antibiotics tested. Pseudomonas isolates showed the highest susceptibility against Gentamicin and polymyxin B, as well as it shows complete resistance to amikacin, cefotaxime, and vancomycin.***

Keyword - Antimicrobial susceptibility, Pseudomonas spp., antibiogram, Kirby -Baur.

Introduction.

Pseudomonas spp. are Gram-negative rod-shaped bacterium, which has a notable capability to adapt and survive in a variety of environments, such as water, soil, clinical settings, hospital, municipal wastewater, and industrial effluents [3]. *Pseudomonas fluorescens* is prominent among PGPR because it induces plant growth as well as control the growth of pathogens [6]. *Pseudomonas aeruginosa* has one of the most extensive ranges of infectivity among all pathogenic microorganisms which causes burn wound infections, nosocomial infections, fulminant infections of bones and joints, endocarditis, meningitis, and pneumonia in cystic fibrosis patients [5]. *Pseudomonas mendocina reported* removing soluble nitrogen in pollutant water [4]. Many *Pseudomonas spp.* are resistant to many antibiotics due to the low permeability barrier due to its outer membrane, presence of multidrug efflux pumps, and its tendency to form a biofilm, making the cells impervious to therapeutic concentrations of antibiotics [3]. The simple and rapid detection of *Pseudomonas spp.* is essential in environmental monitoring and assessment and, by extension, the protection of public health. An antibiogram is a result of in vitro testing sensitivity of bacterial isolates to various antibiotics. The aim of this study was the isolation, biochemical characterization, and conducting an antibiogram study of *Pseudomonas spp.* from various natural sources.

Sample used.

Various samples have collected from natural and industrial sources for research [9]. The samples were as follows: Garden soil sample (location: Ramnarain Ruia college garden, Matunga), Metal contaminated soil sample (location: Western railway workshop, lower Parel), Petroleum contaminated soil sample (location: Hindustan petrol pump, Airoli), Contaminated seawater (location: Marine drive, Churchgate), Dairy effluent sample (Jama masjid dudhnaka, Kalyan), Edible oil-contaminated soil sample (location: Jay malhar vada-pav center, Airoli)

Enrichment and isolation [1].

5 gm of each soil sample and 10 ml of each water sample inoculated in 100 ml of sterile nutrient broth. The inoculated broth incubated at 37^0 C for 24-48 hours. Depending on the development of growth, transferred it to the 50 ml of sterile cetrimide broth, which is a selective medium for *Pseudomonas spp.* and incubated at 37^0 C for 24-48 hours. After that, isolated on sterile cetrimide agar plate *spp.* and incubated at 37^0 C for 24-48 hours. The isolates then further studied for its colony characteristics. Each strain from the different samples subjected to enrichment in sterile cetrimide broth. The culture maintained on sterile nutrient agar slants and incubated at 37^0 C for 24-48 hours. These slants stored in the refrigerator at 4^0 C for culture maintenance [1,9].

Characterization of *Pseudomonas spp.* [1,8]

For confirmation of these isolated strains, the biochemical tests performed, as mentioned in Bergey's manual of systematic bacteriology. It includes catalase test, oxidase test, starch hydrolysis test, haemolysis test, fluorescence test, citrate utilization, triple sugar iron test, motility, glucose utilization test, arginine utilization test, and gelatine hydrolysis test.

Detection of extracellular enzyme production by *Pseudomonas spp.* [2]

Production of proteolytic enzymes was examined on plate count agar (Himedia) containing 10% skim milk powder. After incubation at 37°C for 72 h, plates were flooded with 1 N HCl to observe clearance zones. Lipase producers were screened using tributyrin agar plates. These plates incubated at 30°C for 48 h. The activity of the lipase found as a zone of hydrolysis around the bacterial colonies. The slime production assessed by the Congo red agar method. In this culture was streaked on to Congo red agar containing 37 g/L of brain heart infusion broth (Himedia), 50 g/L of sucrose, 10 g/L of agar, and 0.8 g/L of Congo red. Black colonies on the surface indicated a positive result. Non-slime-producing strains developed red colonies.

Antibiotic susceptibility profile of isolated *Pseudomonas spp.* [3]

Antibiotic susceptibility checked by the disc diffusion method. A loop full of each isolated strain inoculated into 5 mL of normal saline. The suspension of the test isolates adjusted to 0.1 O.D. With the aid of sterile swab, each of the test suspension spread on Mueller-Hinton agar plates. Amikacin, Gentamicin, Polymyxin B, Cefotaxime, and Tetracycline disc placed on a previously swabbed agar plate with isolated isolates. Plates incubated at 37^0C for 24 hours. The inhibitory zone indicates susceptibility toward respected antibiotics.

Result and discussion

Isolation and Identification of Pseudomonas spp.

Various samples analysed for checking the presence of *Pseudomonas spp*. Cetrimide agar used for selective isolation for *Pseudomonas spp.*[1,9], Various isolates observed and named accordingly, such as garden soil- GS1, GS2, GS3; Petroleum contaminated soil sample - PS; Metal contaminated soil sample- MS; Contaminated seawater- SW, Dairy effluent sample- DAIRY, Edible oil-contaminated soil sample- OIL.

Characterization of the isolates.

Samples		**GS1**	**GS2**	**GS3**	**PS**	**SW**	**MS**	**OIL**	**DAIRY**
Morphology									
Colony characteristics		Medium, Irregular, Green, Lobate, Elevated, Mucoid, Translucent, Gram-negative rod.	Medium, Irregular, Green, Undulate, Elevated, Mucoid, Translucent, Gram-negative rod.	Medium, Irregular, Green, Entire, Elevated, Mucoid, Translucent, Gram-negative rod.	Medium, Regular, Green, Elevated, Mucoid, Translucent, Gram-negative short rod.	Medium, Circular, Serrate, Colourless, Elevated, Mucoid, Translucent, Gram-negative rod.	Medium, Regular, Off-white, Elevated, Mucoid, Translucent, Gram-negative short rod	Medium, Regular, Off-white, Elevated, Mucoid, Translucent, Gram-negative short rod.	Medium, Circular, Regular, Off white, Elevated, Mucoid, Translucent, Gram-negative short rod.
Oxidase		+	+	+	+	+	+	+	-
Catalase		+	+	+	+	+	+	+	+
Motility		+	+	+	+	+	+	-	-
Pseudomonas agar plate		+	+	+	+	-	-	-	-
Growth at	**4^0 C**	-	-	+	-	-	-	-	-
	42 ^{0}C	-	-	-	+	-	-	-	-
Gelatine hydrolysis		-	-	+	+	-	-	+	-
Starch hydrolysis		+	-	+	-	-	-	-	+
Nitrate reduction test		+	+	+	+	+	+	+	+

Utilization of l-arginine		-	-	-	+	-	+	+	+
Triple Sugar Iron	**Slant**	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk
	BUTT	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk
	GAS / H_2S	-	-	-	-	-	-	-	-
Simmon citrate		+	+	+	+	+	+	+	+
Hugh & Leiffson medium	**Oxidative**	+	+	+	+	+	+	+	+
	Fermentative	-	-	-	-	+	+	-	-

KEY- - negative; + - positive; ALK – alkaline; GS1, GS2, GS3- Garden soil; PS- petroleum-contaminated soil; SW- contaminated seawater; MS- metal contaminated soil; OIL-Edible oil-contaminated soil; DAIRY- dairy effluent contaminated soil.

- **Oxidase test** - blue colored colonies, produce cytochrome c oxidase that shows a positive oxidase test.
- **Catalase test** - Positive strains possess catalase enzyme breaks hydrogen peroxide (H_2O_2) into the water and release O_2, giving bubbles on the slide.
- **Pseudomonas agar plate** - pigment produced from the colonies of pseudomonas diffuses into the agar and shows green-yellow fluorescent coloration due to the production of small amounts of pyocyanin.
- **Starch hydrolysis test** - clear zone around colonies in starch agar plate after iodine treatment indicates their ability to digest the starch by alpha-amylase.
- **Gelatine hydrolysis test** - positive strains produce gelatinase (proteolytic enzyme) that liquefy gelatine.
- **Nitrate reduction test**- determines the production of an enzyme called nitrate reductase, which makes nitrate reduced to nitrite (NO_2), Formed nitrous acid reacts with sulfanilic acid and naphthylamine, which form a red color.
- **Motility Test**- Overnight incubation all the test tubes inoculated with isolated strains observed for hazy appearance, which was the indication of the motile organism.
- **Arginine utilization -** enzyme breaks the bond holding the carboxylic group and amino group, which causes the pH to goes up, changing the indicator Bromo-cresol purple to turn purple gives positive arginine hydrolysis test.
- **Triple sugar ion test -** The negative results were indicated by alkaline production (change in color from red to pink) indicated no sugar production, and the color of the medium was not turning into black; indicate the absence of H2S production.
- **Citrate utilization** – Positive strains utilize Ammonium dihydrogen phosphate and Sodium Citrate as their primary sources of nitrogen and carbon produces a color change from green (neutral) to blue (alkaline).
- **Glucose utilization** – Yellow color indicates oxidative and fermentative metabolism.

Moreover, From the results obtained from the various biochemical test performed and analyze with the data mentioned in reference paper[4-8], this data concluded that isolated strains from garden soil, petroleum-contaminated soil, edible oil-contaminated soil, metal contaminated soil, and contaminated seawater, possess similar characteristics like *Pseudomonas fluorescence, Pseudomonas aeruginosa, Pseudomonas alkaligens, Pseudomonas mendocina, Pseudomonas putida* Respectively. DAIRY, GS1, GS2, isolates obtained from dairy effluent and garden soil, although belonging to *Pseudomonas* genera, could not be identified by the biochemical test.

Table no. 1 Biochemical result presenting identification of isolates.

Fig 1. Biochemical results of isolates.

Extracellular enzyme production by isolates.

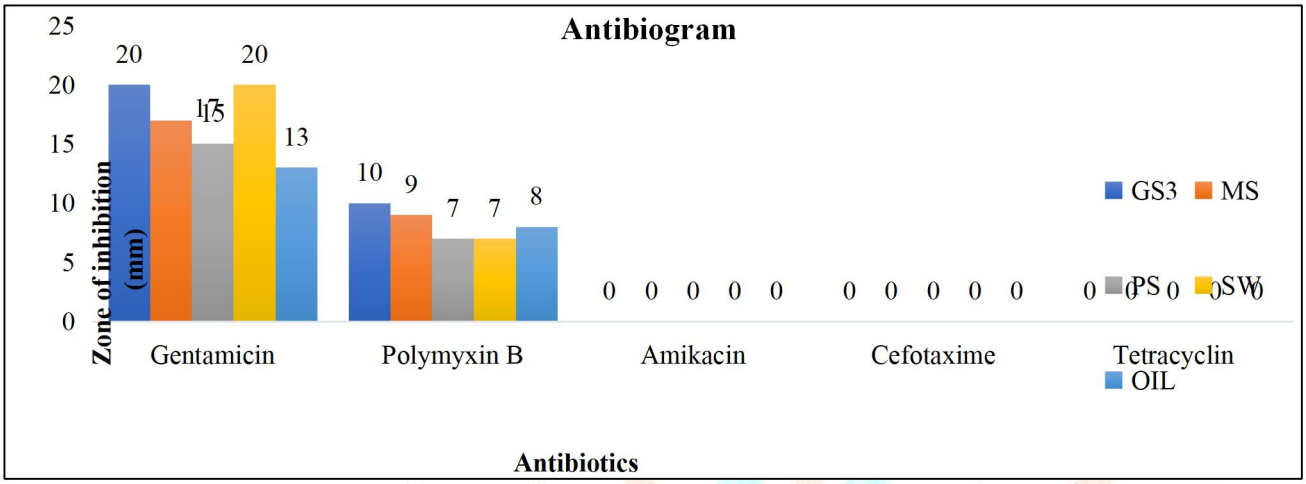
In this study, most of the isolated *Pseudomonas spp*. found to produce extracellular enzymes such as protease, lipase, which is responsible for the degradation of proteins and lipids. None of them was a β-Lactamase producer.

	GS3	MS	SW	OIL	PS
Protease	+	+	+	+	+
Lipase	+	+	+	+	+
B-lactamase	-	-	-	-	-
Slime production	+	+	+	+	+

Antibiotic susceptibility test

The data suggests that all isolated *Pseudomonas* strains were susceptible to antibiotics such as tetracycline, cefotaxime, and amikacin. Gentamycin and B were microbicidal against isolated *Pseudomonas* strains. Our study agreed with the Beshiru, Abeni, and Etinosa O.

Igbinosa research study; the capacity of the resident organisms to proliferate in such an environment gives it an inherent ability to resist the action of various antimicrobials [3]. It could, in turn, result in the selective proliferation of resistant strains in the environment. Therefore, continuous monitoring and assessment of the environment are essential. The presence of multidrug-resistant *P. aeruginosa* in various environments increases the possibility of these organisms to disseminate potential resistance and virulence genes within bacterial populations [3].



Graph 1. Antibiotic Susceptibility Pattern against isolated *Pseudomonas spp.*

Conclusion

In this study, various strains of *Pseudomonas spp*. from various natural sources identified. None of them was a β-lactamase producer. Gentamycin and polymyxin B found to be the most effective drugs against isolated *Pseudomonas spp*. However, all strains were resistant to amikacin, cefotaxime, and vancomycin. Thus, with the available shreds of evidence from the current literature, there is enough reason to believe that *Pseudomonas species* are associated with human disease. Therefore, careful attempts are necessary for their isolation and identification from various sources, and they should not discard as contaminants or non-pathogens.

Acknowledgments- No

Conflict of interest -No

Funding Sources- No

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