



## Isolation and Identification of *Pseudomonas aeruginosa* from different sources (soil, wound, urine) and Checking its MIC with various Antibiotics

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### Abstract

*Pseudomonas* is a group of bacteria found in soil, water, skin flora and most man-made environments throughout the world. *Pseudomonas* infections may cause disease, generalized inflammation and sepsis. The targeted organism for this organism are the lungs, the urinary tract, and kidneys, the result can be fatal. It can also be found on medical equipment. *Pseudomonas* is well known to be animal and plant pathogen. It is also known to cause food spoilage. Over 50 different types of *pseudomonas* can infect humans, but most infections are caused by *pseudomonas aeruginosa*, which is known for its role as an opportunistic human pathogen. In the present study a total of 10 soil samples, 25 samples of urine and 25 samples of wound were collected from Health centre, Osmania University for the isolation of *pseudomonas spp.* All the samples were inoculated onto selective medium "Kings Media" for isolation of all *pseudomonas spp.* and Cetrimide agar for isolation of *pseudomonas aeruginosa*. A total of three *pseudomonas aeruginosa* isolates were selected for further studies taking one *pseudomonas aeruginosa* from each of the source. All the three were identified by biochemical characteristics. For all the three strains Antibiotic susceptibility testing (AST) was carried out to determine the antibiotic sensitivity and resistant in plate culture conditions. Presence of plasmid DNA indicated the possibility of antibiotic resistance in *pseudomonas spp.* and also MIC (Minimum Inhibitory Concentration) for three strain of *pseudomonas aeruginosa* was detected.

### Keywords

*Pseudomonas*, Cetrimide agar, Kings Media, MIC, Opportunistic Pathogen.

### Introduction

*Pseudomonas* is a genus of gram-negative, aerobic gammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species [1] The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches [2] Their ease of culture in vitro and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for scientific research;

the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant Pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*. Because of their widespread occurrence in water and plant seeds such as dicots, the *Pseudomonads* were observed early in the history of microbiology. The generic name *Pseudomonas* created for these organisms was defined in rather vague terms by Walter Migula in 1894 and 1900 as a genus of Gram-negative, rod-shaped and polar-flagellated bacteria with some sporulating species [3, 4] the latter statement was proved incorrect and was due to refractive granules of reserve materials. Despite the vague description, the type species, *Pseudomonas pyocyanea* (basonym of *Pseudomonas aeruginosa*), proved the best descriptor [5]

### *Pseudomonas aeruginosa*

Is a common Gram-negative bacterium that can cause disease in animals, including humans. It is citrate, catalase, and Oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the, the lungs urinary tract, and kidneys, the results can be fatal [6] Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infection in hospitals and clinics. It is implicated in hot-tub rash. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oil spills [7, 8]

### Antibiotic resistance in *P. aeruginosa*

*P. aeruginosa* is one of the main organisms responsible for drug-resistant nosocomial infections, and is one of the leading causes of bacteremia and pneumonia in hospitalized patients. In addition being intrinsically resistant to several antimicrobial agents, *P. aeruginosa* can easily develop resistance to all conventional antipseudomonal antibiotics via different mechanisms (9).

The amino glycosides inhibit protein synthesis by binding to the 30S subunit of the ribosome. Quinolones bind to the A subunit of DNA gyrase enzyme, which maintains the ordered structure of the chromosome inside the cells. The  $\beta$ -lactams inhibit the peptidoglycan-assembling trans peptidases located on the outer face of the cytoplasmic membrane. Finally the polymyxins bind to phospholipids in the cytoplasmic membrane, destroying its barrier function. There are three basic mechanisms by which

*P. aeruginosa* resists the action of the above antimicrobial agents: restricted uptake and efflux; enzymatic drug inactivation and mutations in the targets (10).

**Materials and Methods**

Three different sources (soil, wound and urine) were used in this study for isolation of pseudomonas.

**Soil sample collection**

For bacterial isolation, 10 different soil samples were collected from five locations (2 samples from each source), Department of Microbiology, Genetics, Landscape garden, Arts College, Law college of Osmania University. One gram of soil was collected and plating was done on kings media using serial dilution method. In this research, nutrient agar (Hi-media) medium was used for bacterial growth. 28 g of nutrient agar was dissolved in 1000 ml distilled water and sterilized in autoclave for 15 min at 121°C. Inoculation was done using 0.1 ml of  $10^{-5}$  dilution of soil suspension and spreaded over the nutrient agar plates using sterilized L shaped glass rod.

**Wound Sample Collection**

A total of 20 samples were collected from wounds of different patients using sterilized swabs. The swabs were transferred to empty sterilized tubes and immediately processed by streaking onto NA plates.

**Urine sample collection:**

A total of 20 Samples from patients of urinary tract infections (UTI) were collected from Health centre, Osmania University. The samples were collected in sterilized vials and immediately processed.

**Inoculation:** 0.1 ml of  $10^{-2}$  and  $10^{-3}$  dilution of soil suspension was poured and spreaded over the nutrient agar plates by using sterilized L shaped glass rod as mentioned above. After obtaining colonies pure colonies were transferred onto Kings Medium.

**Results and Discussion**

**Isolation of bacteria from different sources**

A total of 60 samples, 10 from soil, 25 from wound and 25 from UTI were collected for isolation of pseudomonas spp. Spread plate method was performed for both soil and urine samples, the

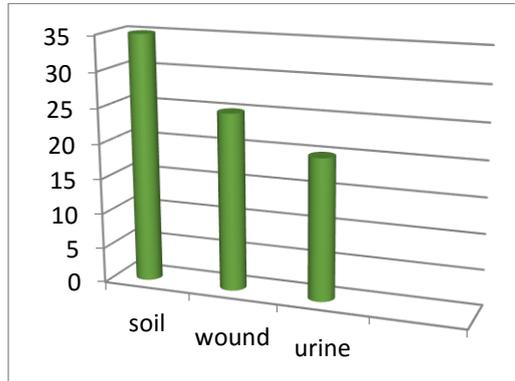
bacterial colonies obtained were counted and the data is represented as CFU/ml of sample (Table 1).

S. No	Soil	Source	CFU (Bacteria)
1.	SS1	Dept. Microbiology	$4.8 \times 10^6$
2.	SS2	Dept. Microbiology	$4.9 \times 10^5$
3.	SS3	Dept. Genetics	$3.3 \times 10^3$
4.	SS4	Dept. Genetics	$4.8 \times 10^5$
5.	SS5	Landscape Garden	$4.2 \times 10^8$
6.	SS6	Landscape Garden	$4.9 \times 10^7$
7.	SS7	Arts College, OU	$4.3 \times 10^6$
8.	SS8	Arts College, OU	$3.5 \times 10^6$
9.	SS9	Law College, OU	$1.7 \times 10^6$
10.	SS10	Law College, OU	$1.8 \times 10^6$

**Table 1: Colony forming units (CFU/ml) of bacteria from soil sample**

The above table shows the number of colony forming units obtained for each sample collected and plated.

**Figure 1. Number of bacterial isolates from different sources**



**Figure 2: Appearance of pseudomonas type of colonies on N.Agar**

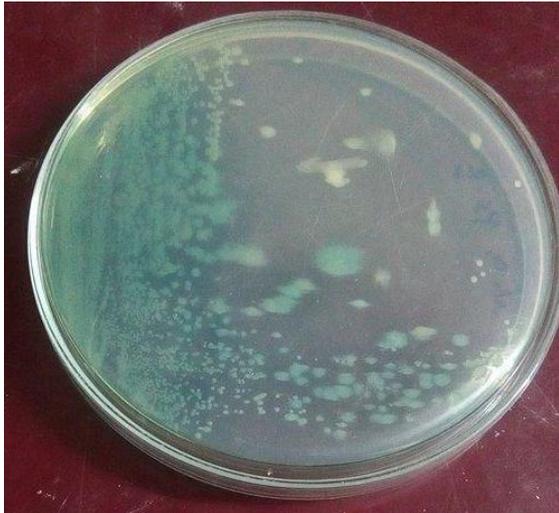
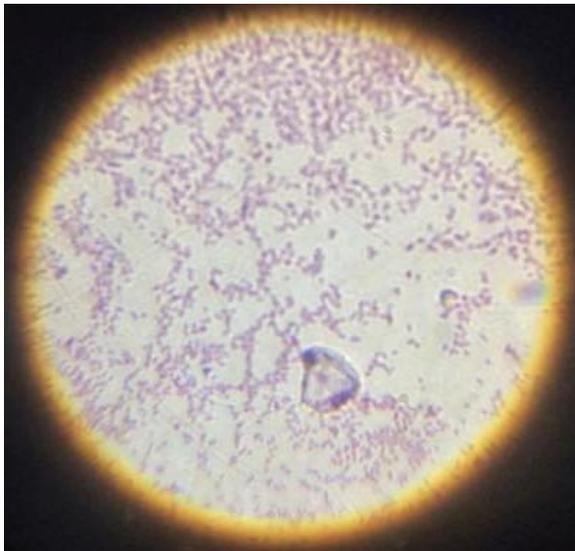


Figure 3: Gram negative pseudomonas



All the three isolates were tested for different biochemical properties for confirmation of the genus pseudomonas. Parameters like catalase and oxidase

were tested (glucose, lactose, sucrose,) and negative production reveals that all these three isolates are *pseudomonas aeruginosa*

Table 2: Biochemical tests performed in this study

Test	Bacterial isolates		
	<i>p.aeruginosa</i> (1)	<i>p.aeruginosa</i> (2)	<i>p.aeruginosa</i> (3)
GS	-	-	-
Indole	-	-	-
MR	+	+	+
VP	-	-	-
Citrate	+	+	+
Urease	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
Coagulase	+	+	+
Haemolysis	+	+	+
Nitrite test	+	+	+

In the above table the number coding given are detailed below: 1. Soil, 2.Wound, 3. Urine  
 GS=Gram Stain, MR=Methyl Red, VP=Vogues Proskeur, A=Acid Production

Based on the above biochemical tests performed, it was observed that all the three isolates showed positive for genus *pseudomonas* and further clarification on spp. was made due to positive catalase, coagulase and hemolysis. This observation revealed that all the three isolates belong to *pseudomonas* group.

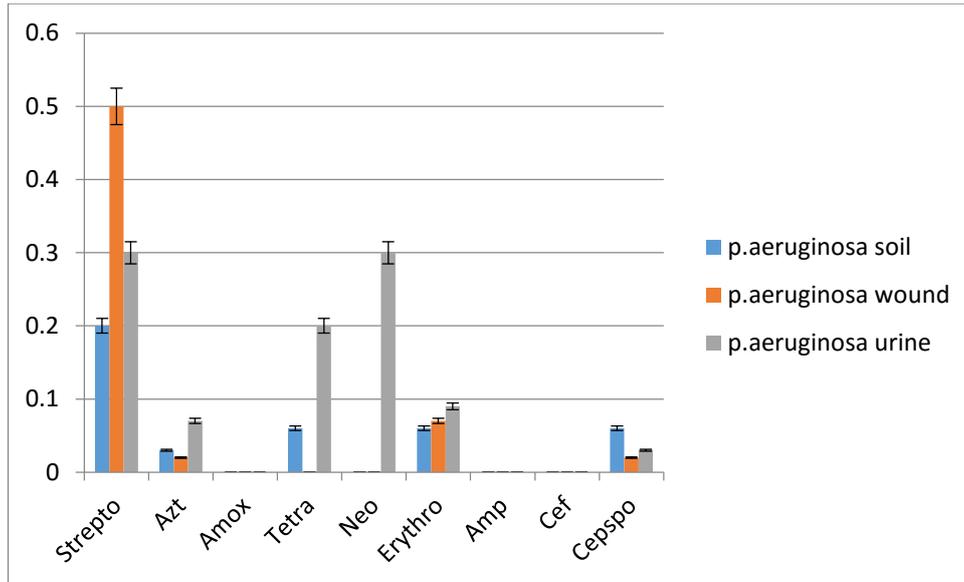
Table 3: MIC (Minimum Inhibitory Concentration) of the different antibiotics against *pseudomonas spp.*

Source	Bacterial isolate	Antibiotics used in the study (mgml <sup>-1</sup> )								
		Strepto	Azt	Amox	Tetra	Neo	Erythro	Amp	Cef	Cepspo
Soil	<i>P.aeruginosa</i>	0.2	0.03	R	0.06	R	0.06	R	R	0.06
Wound	<i>P.aeruginosa</i>	0.5	0.02	R	R	R	0.07	R	R	0.02

Urine	<i>P.aeruginosa</i>	0.3	0.07	R	0.2	0.3	0.09	R	R	0.03
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Strep= Streptomycin, Azt=Azithromycin, Amoxicillin, Tetra=Tetracyclin, Erythro=Erythromycin, Amp=Ampicillin, Cef=Cefaxm, Cepspos=Cephalosporins, neomycine

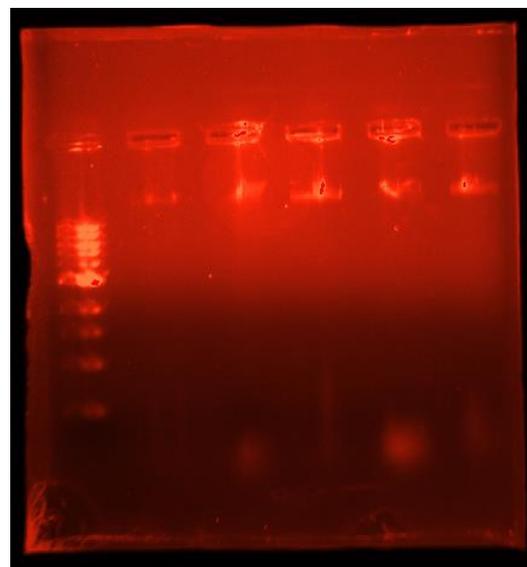
**Figure (4) Antibiotic**



**Plasmid DNA**

Antibiotic resistant pattern is attributed to the presence of plasmid in all the three pseudomonas isolates. However, further studies of plasmid curing are required for confirming the same. Appearance of three bands indicates plasmid DNA and presence of supercoiled, nick and circular plasmid is seen in figure 5.

**Figure 5: Plasmid DNA from pseudomonas soil, pseudomonas wound and pseudomonas urine (well 1, 2, 3, 4, 5). Well1-Marker**



**Discussion**

Microscopically *pseudomonas aeruginosa* are motile (one or more polar flagella), rod shaped and aerobic, Gram-negative, non-fermentative bacteria. The typical bacterial size is 0.5 – 1.0 x 1.5 – 5.0 μm.

Hence, this study focused on isolation and characterization of *pseudomonas* from different

sources such as soil, wound and urine provided us the information about the variability in antibiotic resistance and susceptibility and variation in minimum inhibitory concentration (MIC) of the three *pseudomonas aeruginosa* (from wound), *pseudomonas aeruginosa* (from soil) and *pseudomonas aeruginosa* from UTI patient samples showed resistant to Cefaxm, Amoxicillin, Ampicillin, and Neomycin except urine. It is sensitive to Neomycin the best sensitive to Cephalosporins for all isolates obtained from different sources. The work can further be extended in isolation of plasmid from *p. aeruginosa* from several other sources.

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