



## The Isolation of *Pseudomonas aeruginosa* From Septic Sore Using Some Biological Tests.

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### Abstract

*Investigations on the isolation and confirmation of *Pseudomonas aeruginosa* from the septic sore of an accident patient of the University College hospital, Ibadan, using some biological tests was carried out. The organism was found to ferment some sugars such as maltose, fructose and sucrose. However, it did not ferment glucose and lactose. It was also sensitive to only a particular antibiotic, which was Ciprofloxacin and was resistance to 9 others which were; Augmentin, Ceftriazone, Tetracycline, Nitrofuranton, Gentamycin, Cotrimozazole, Ofloxacin, Amoxicillin, and Pefloxacin. Some biochemical tests such as Gram staining, Catalase test, Oxidase test, Indole test, Starch hydrolysis and Gelatin utilization were carried out on the organism. Gram's staining reaction and Indole test gave negative results, while the catalase, starch hydrolysis, gelatin utilization and Oxidase tests showed positive results. *Pseudomonas aeruginosa* from septic sore has been found to be highly resistant to antibiotics, thus, proper care and precautions should be taken by individuals with sores.*

**Key words:** Lippolysaccharide, Inoculation, Biochemical test, Septic sore, Ubiquitous.

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### Introduction

*Pseudomonas aeruginosa* is a micro organism that belongs to the Kingdom Proteobacteria and the Family Pseudomonadaceae (Ryan and Ray 2004). They are obligate aerobes with polar flagellation. Three species are of considerable pathogenic significance in animals and *P. aeruginosa* is one of them (Matthijs et al. 2007). Generally, *Pseudomonas* species are ubiquitous, they can be found in water, soil, vegetation, sewage and also in the intestinal tract (Ryan and Ray 2004). *P. aeruginosa* is frequently found in hospital environment especially in moist places like sinks, bowls, drains and cleaning buckets (O Marhoney et al. 2005).

According to (Cornelius 2008) *P. aeruginosa* is motile by means of a single polar flagellum. Thus, it can live in a sessile biofilm form, or it can live in a planktonic form as a free swimming cell. *P. aeruginosa* is recognized by its yellowish green pigmentation on nutrient agar. *P. aeruginosa* is notorious for its resistance and is therefore a dangerous and dreaded pathogen (Huertas et al. 2006). The resistance

of *P. aeruginosa* is due to the permeability barrier afforded by its outer membrane lippolysaccharide (Krieg and Noel 1984). Its tendency to colonize surfaces in the biofilm form makes the cells impervious to therapeutic concentrations of antibiotics (Sepulveda Torress et al. 1991). *Pseudomonas* infection may be seen as composed of 3 distinct stages which includes; bacteria attachment and colonization, local invasion and disseminated systemic disease (Krieg and Noel 1984). The most selective antibiotics are those that interfere with the synthesis of bacterial cell wall and examples include; Penicillin, Spectinomycin, Tetracycline and many others that inhibit protein synthesis by binding with the prokaryotic ribosome (Ryan and Ray 2004). Studies have shown that *P. aeruginosa* is resistant to most of the commonly used antibiotics and this is an inevitable consequence of misuse of antimicrobial agents all over the world (Krieg and Noel 1984). Thus the main thrust of this study is to isolate, characterize and test the antibiotic sensitivity of *P. aeruginosa* from a septic sore.

### Objectives of this work include;

- The isolation in a pure culture of *P aeruginosa* from septic sore
- Characterization by morphological, cultural and biochemical attributes.
- Antibiotic sensitivity test on the isolate.

### Materials and Method.

The sample used in this investigation was collected from septic sore of an accident patient of the University College hospital, Ibadan. The collection was done in an aseptic condition.

#### Preparation of the Medium

The medium used is the Lab M nutrient agar. It was prepared aseptically according to the manufacturers' directions. The media was prepared by dissolving fourteen grams (14g) of nutrient agar powder in 500ml of distilled water, the mixture was then autoclaved.

#### Isolation of the Organism.

The sample after collection aseptically, was streaked on the surface of the nutrient agar slant using a sterile swab under aseptic condition. After the streaking process, the slants were incubated at 37°C for 24 hours. Purification of the organism was done by repeated sub-culturing on fresh nutrient agar slants.

### Confirmatory Test to Identify the Isolate.

#### Gram Staining Reaction

A smear from the 24 hour old culture was made on a clean grease-free slide and heat fixed. Crystal violet was initially used to flood the slide after which Grams iodine was applied and then washed off after one minute. Acetone was used to decolorize the smear and washed under slowly running water. Safranin solution was used to counter-stain the smear for 30 seconds; it was then washed off using water. The slide was air dried; oil immersion (a drop) was added and covered with a cover-slip. The morphology of the cells was observed under the microscope. A pink coloration indicates that the organism is Gram positive.

#### Catalase Test.

A drop of hydrogen peroxide was placed on a clean slide. A sterile inoculating loop was used to collect some of the isolate and was placed on the hydrogen peroxide. Effervescence indicates that the organism is catalase positive.

#### Oxidase Test.

A sterile swab was used to collect little of the isolate on a clean slide. A drop of oxidase reagent was placed on the isolate. Purple coloration indicates that the organism is oxidase positive.

#### Indole Test.

Sterilized tryptone water in test tubes was prepared in duplicates and each tube inoculated with the organism. Incubation was done for 72hrs at 35°C, Kovacs reagent was added to each culture. The observation of a red ring after

introducing kovacs reagent into the media indicates that the organism is indole positive.

#### Sugar Fermentation.

Five (5) different sugars were involved in this test which includes; maltose, sucrose, glucose, fructose and lactose. The organism was introduced into an already prepared sugar in test tubes using an inoculating loop. The tubes were covered with sterile cotton wool and were left under room temperature for observation. Color change observed in tubes indicates that the organism fermented the sugar.

#### Starch Hydrolysis.

0.01g of the Lab M starch was added to 1.12g of nutrient agar. 40ml of water was added to the mixture. The media was then autoclaved and allowed to cool for a few minutes after which it was poured into plates and swerved gently for equal spread round the plate. The plates were left to solidify. A 24hour old culture of the organism was collected, streaked on the media and incubated at 35°C for 24hrs. After incubation, the plate was flooded with Grams iodine. If a clear zone round the growth of the organism is observed, it indicates that starch was hydrolyzed by the organism.

#### Gelatin Utilization.

To 100ml of distilled water, 0.28g of nutrient agar powder and 0.15g of gelatin powder were added into sterile test tubes. The media was autoclaved and allowed to cool for a few minutes after which it was inoculated with 24hr old culture. The incubation was done at 35°C for 48hrs. After incubation, the tubes were transferred into a refrigerator until it was completely chilled. If the media remains liquefied even after refrigeration, it indicates that the result is positive.

#### Sensitivity Test.

0.76g of the Lab M Muller Hinton agar was added to 20ml of distilled water in a plate and then autoclaved. A sterile swab was used to collect the organism and inoculated on the media. A sterile forceps was used to collect a sensitivity disc and was placed in the centre of the media. The plate was incubated at 35°C for 24hrs after which observations were recorded. If a clear zone around an antibiotic on the sensitivity disc is observed, it indicates that the organism is sensitive to that particular antibiotic.

### Results.

Table 1: Results of Biochemical Tests

Tests	Results
Gram Staining Reaction	Negative
Catalase Test	Positive
Oxidase Test	Positive
Indole Test	Negative
Starch Hydrolysis	Positive
Gelatin Utilization Test	Positive

As shown on table 1, the Gram staining reaction was negative, indicating that safranin was retained on the smear.

The indole test was negative. The absence of the formation of a red ring on the media after a drop of Kovacs reagent was introduced showed that the organism involved is indole negative. The catalase test gave a positive result. Effervescence was observed, indicating the presence of catalase enzyme. The result of the oxidase test was also found to be positive. The color of the organism changed to purple after 30secs of observation. For the starch hydrolysis reaction, at flooding the already inoculated plate with Grams iodine, a clear zone is observed round the growth of the organism which indicated that starch was hydrolyzed by the organism. Also for the gelatin utilization test, the media remained liquefied even after refrigeration giving a positive result

Of all the 5 sugars studied, lactose and glucose gave a negative result indicating that the organism did not ferment the sugar, thus no color change was observed. However, maltose, fructose and sucrose gave positive results, showing that the organism fermented these sugars thus bringing about the changes from red to yellow.

The organism was resistant to 9 antibiotics on the sensitivity disc, which were; Augmentin, Ceftriazone, Tetracycline, Nitrofurantone, Gentamycin, Cotrimoxazole, Ofloxacin, Amoxicillin, and Pefloxacin but was sensitive to only one which was Ciprofloxacin. Also, due to the fact that the organism was Gram negative, a Gram negative antibiotic disc was used. The organism was thus found to be highly resistant to antibiotics

### **Discussion.**

The sensitivity test conducted by (Anzai et al. 2000) showed that *Pseudomonas aeruginosa* was sensitive to two antibiotics; Ciprofloxacin and Gentamycin. However in this study it was observed that the organism was sensitive to only one antibiotic, Ciprofloxacin. Also, (Krieg Noel et al. 1984) found out that the studied organism fermented not only maltose and fructose but also fermented glucose. However, glucose was not fermented by the organism in this study. Pathogenesis may in part be due to the proteins secreted by *P. aeruginosa* although colonization unusually precedes infection by *P. aeruginosa*, the exact source and mode of transmission of the pathogen are often unclear due to their ubiquitous presence in the environment (Madigan 2005). Infections caused by *Pseudomonas* sp. includes; Endocarditic, Pneumonia, Infections of the Urinary tract, Central Nervous System, Wounds, Eyes, Ears, Skin and Skeletal system(Nametal 2003). Of the 2 million nosocomial infections recorded recently, 10% were caused by *P. aeruginosa*. Several factors make the organism highly resistant. These include; the ability to stick to cells, minimal food requirement, resistance to many antibiotics, production of protein that damages tissue and a protective outer coat (Onaca et al.2007). *Pseudomonas aeruginosa* from septic sore has been found to be highly resistant to antibiotics,

thus, proper care and precautions should be taken by individuals with sores.

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