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Isolation, Screening and Antibiotic Sensitivity of *Pseudomonas* species from Kelana Jaya Lake Soil in Selangor Malaysia

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Abstract:

Pathogenic microorganisms from hospitals, communities, and the environment remain great threats to human health. The increasing concern about antibiotic resistance has also necessitated the search for robust alternatives. Therefore, this study aims to isolate, screen and evaluate the antibiotic susceptibility of Pseudomonas aeruginosa isolated from a soil sample taken from northern, western and eastern parts of Kelana Jaya Lake against four antibiotics (gentamycin, tetracycline, ampicillin, and penicillin) on a Mueller-Hinton Agar media plate. Pseudomonas identification was done by using API 20 kit. Disc diffusion was employed as well as the oxidase test. From the positive oxidase result, the isolated bacteria were identified as Burkholderia cepacia (97.6% ID), Pseudomonas aeruginosa (99.5-99.9% ID), and Pseudomonas fluorescent (75.9% ID). Only Pseudomonas aeruginosa isolates were further evaluated for antibiotic susceptibility tests. The result showed that P. aeruginosa was susceptible to only three antibiotics (gentamycin, tetracycline, and penicillin) showing a clear zone of inhibition while it was resistant to only ampicillin with no zone of inhibition. Soil isolates are potential sources for the development of effective antibiotics against resistant bacteria.

Key words: Antibiotics, Bacterial resistance, Pseudomonas spp.,Sensitivity, Soil.

Introduction:

Pseudomonas is a rod-shaped Gram-negative that is commonly found in water and soil environments. It can catabolize different types of organic and natural compounds and thus capable of inhabiting various ecological niches (1-3). Several *Pseudomonas* species play beneficial roles in the ecosystem while others are, however, pathogenic with difficult-to-control pathogenicity and responsible for various infections such as lung infections, skin infections, urinary tract infections as well as upper respiratory infections (4,5). As an opportunistic pathogen, *Pseudomonas aeruginosa* has a remarkable capacity to cause diseases in susceptible hosts. It is the major bacterial pathogen that colonizes cystic fibrosis patients (6) and one of the most common infectious agents in nosocomial infections, patients with a severe burn, cancer, transplantation, AIDS, bronchiectasis, chronic lung infection, urinary tract infections, kidney infections, and other immuno-compromising diseases (3).

P. aeruginosa is also known for its unique capability to develop resistance against most antibiotics, with multi-drug resistant strains commonly isolated from infected patients (7 , 8). Previous studies have suggested that antibiotic resistance could be developed as a result of several mechanisms, including antibiotic-modifying enzymes (through acetylation, phosphorylation, and

adenylation) and intrinsic resistance mechanisms (such as decreased outer membrane permeability and upregulation of multidrug efflux pumps) (9 , 10).

The emergence of continuously rising antimicrobial resistance has greatly challenged and reduced the effectiveness of most clinical antibiotics. The genus *Pseudomonas* is heterogeneous and one of the most virulent pathogens, in terms of antibiotic resistance (9 , 11).

On the other hand, microorganisms produce antibiotics as secondary metabolites (12). Therefore, the chain of novel antibiotics as an alternative and better chemotherapeutic agents could be enhanced through the isolation of antibiotics from these microorganisms (13). The rapidly increasing spread of multi-drug resistant pathogens which cause several life-threatening diseases is majorly responsible for the snowballing of the demand for new antibiotics (14-16). Thus, this research is aimed to isolate, characterize, and identify *Pseudomonas* species from the soil of Kelana Jaya Lake.

Materials and Methods:

Soil Sample

Soil sample procedure was carried out according to the literature with slight modification (17). Briefly, three soil samples were taken from the northern, western, and eastern parts of Kelana Jaya Lake. The samples were taken aseptically, kept in containers, and were stored in the refrigerator until further use.

Bacterial isolation

For bacteria isolation, 1 g of moist soil sample was added and suspended in 9 mL of distilled water to prepare a microbial suspension. The solution was agitated on a vortex for 15 minutes. About 1 mL of the sample solution was transferred by using pipette into 10^{-1} serial dilution which contains an additional 9 mL of distilled water. The tube was mixed properly. The serial dilution from 10^{-1} up to $10^{-5}$was prepared. Then, 1 mL aliquot of different dilutions was added to sterile Petri dishes (triplicate for each dilution) to which around 25 mL of sterile molten (45 °C) Mueller-Hinton agar media was added after being autoclaved and allowed to cool down. The Petri dishes were, then, incubated at 28 ± 2 °C for 24 -72 hours for colony formation. Observations were recorded daily (18).

Colony purification

To obtain pure cultures of each isolated colonies, the streak plate method was performed. Every single colony was selected and streaked on MacConkey agar plate. The plate was incubated at 28 ± 2 °C for 24 -72 hours until the clear colonies appear.

Bacterial identification and characterization

The Gram staining method was carried out to characterize the isolated bacteria. The characteristics such as color, elevation, pigmentation, shape, size, surface, margin, odor, etc., of the *Pseudomonas* spp. on the media were recorded (19).

The oxidase test was carried out following the manufacturer's instructions provided in the kit. The results were recorded carefully on the result sheet for the final bacterial profile (API20E) (Figs. 1, 2 and 3).

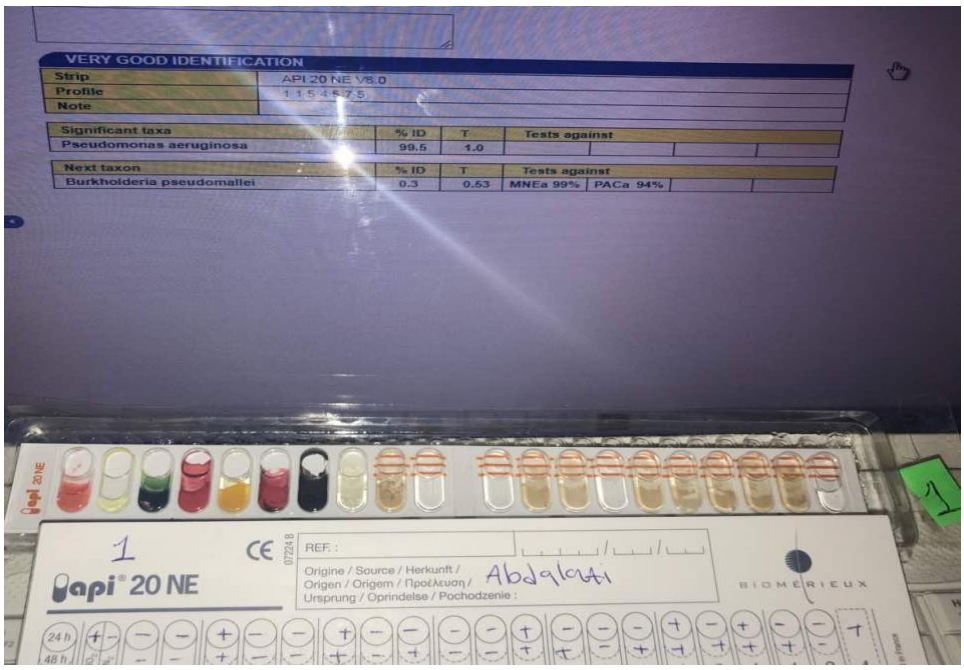

Figure 1. Identification of API 20 NE result as *Pseudomonas aeruginosa*

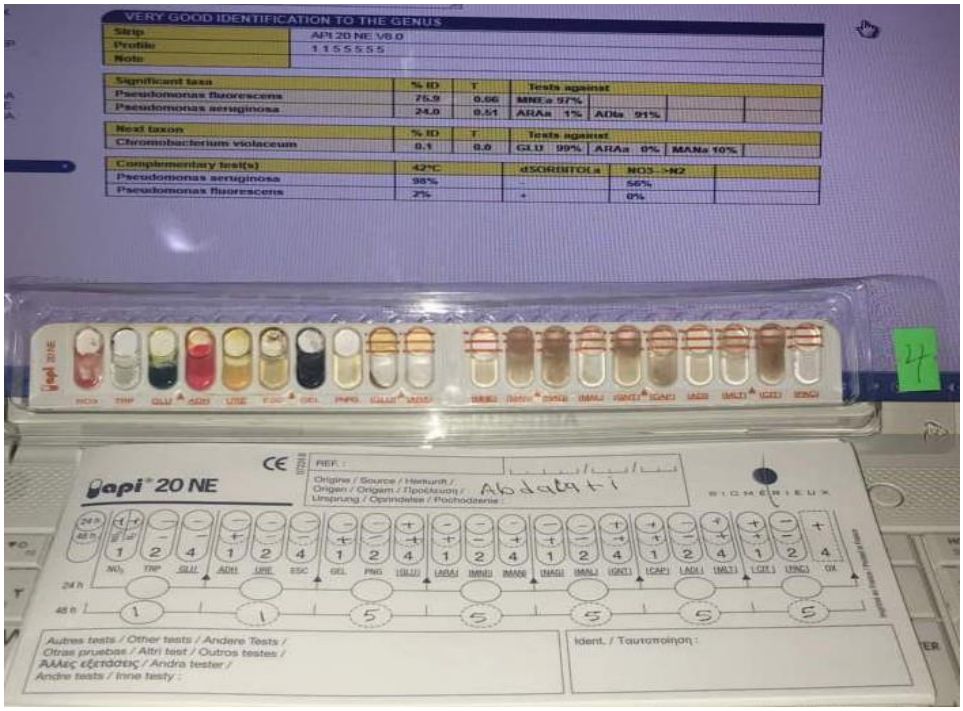

Figure 2. Identification of API 20 NE result as *Pseudomonas aeruginosa and Pseudomonas fluorescens*

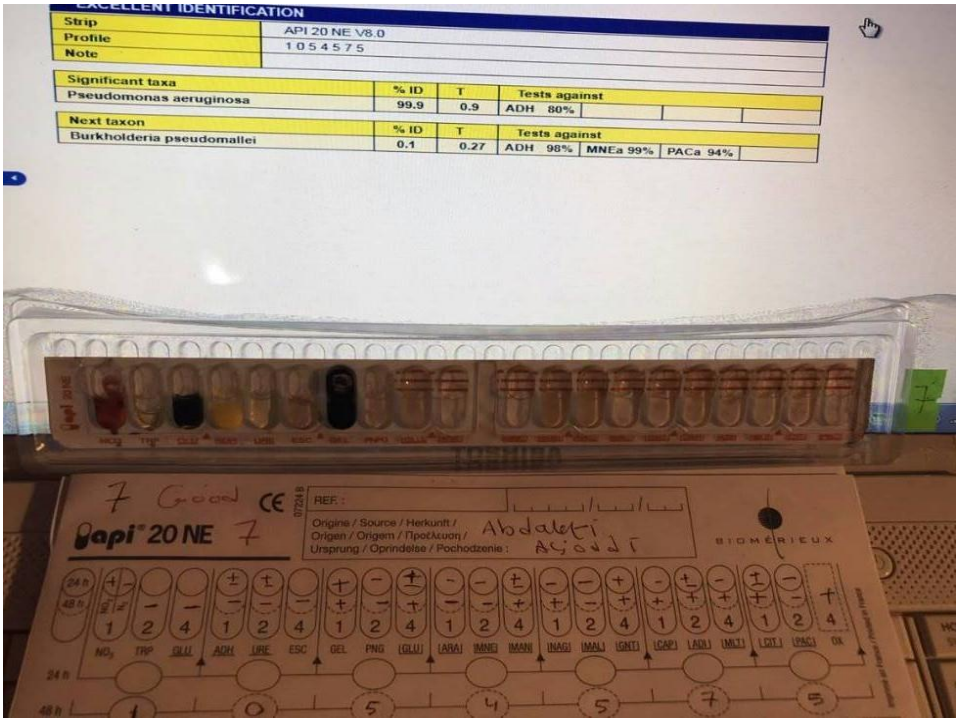

Figure 3. Identification of API 20 NE result as *Pseudomonas aeruginosa*

Disc diffusion method

The antibacterial sensitivity was performed using the disc diffusion method. Admittedly, only the *Pseudomonas aeruginosa* isolates were further evaluated for antibiotic susceptibility tests. About 20 mL of sterilized Muller Hinton (MH) was poured into a sterile petri dish. Then, about 100 µL of 24 hours old culture of *Pseudomonas* spp suspension was spread on MH agar plates after solidification. Standard discs of antibiotics (gentamycin, tetracycline, ampicillin, and penicillin), that were available in our laboratory, were used to analyze the antibiotic sensitivity against *Pseudomonas* spp. (20). Distilled water was used as a negative control. The plates were prepared in triplicates. Then, the plates were incubated at 37°C for 24 hours. After incubation, the inhibitory zones diameter formed around each well were observed, measured in mm and recorded, according to the Clinical &

Laboratory Standards Institute (CLSI Catalog 2019) guidelines.

Statistical Analysis

The statistical packages of SPSS version 22 were used for the statistical analysis. A significant difference was determined at $p<0.05$ with a one-way ANOVA test for the antibacterial sensitivity evaluation. The results were also analyzed using Tukey's HSD posthoc test.

Results:

Different bacteria colonies were successfully screened and isolated (Fig. 4). Morphological characterizations of isolated strains were done by Gram's staining.

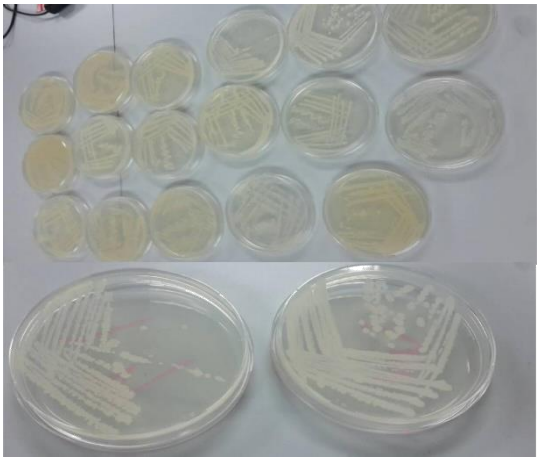

Figure 4. Screened and isolated bacteria colonies

The staining showed that the isolates were Gram-negative. The analysis of API kit results was done in the API WEB system server. The oxidase test result was positive. The isolated bacteria were identified as *Burkholderia cepacia* (97.6% ID), *Pseudomonas aeruginosa* (99.5-99.9% ID), and *Pseudomonas fluorescent* (75.9% ID) (Fig. 5) .

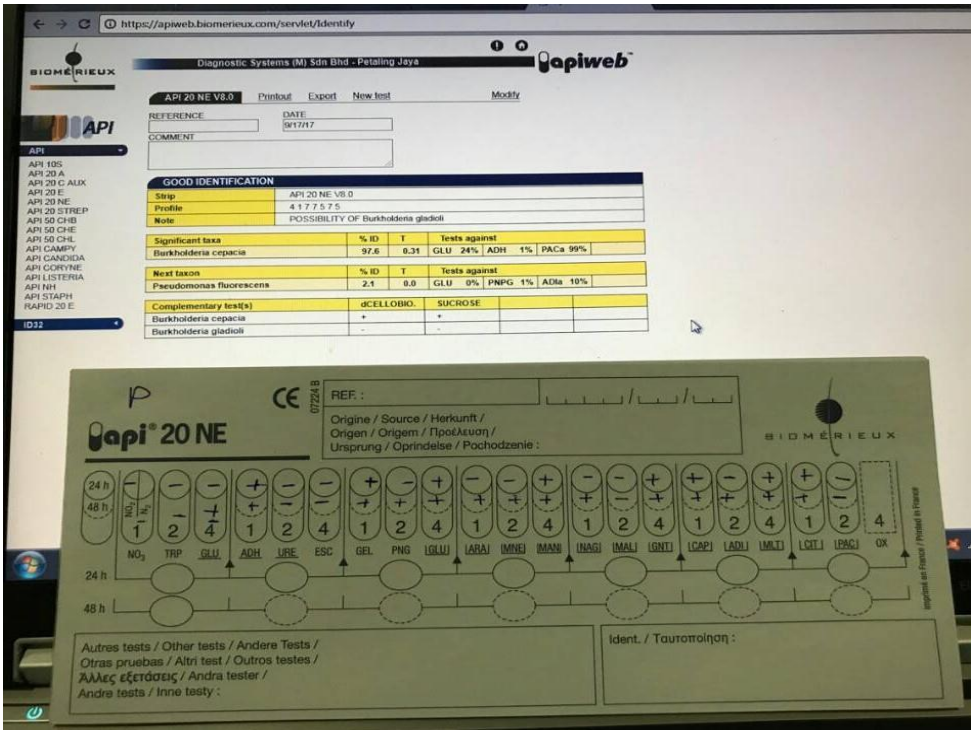

Figure 5. Identification of API 20 NE result as *Burkholderia cepacia*

The antibacterial sensitivity with antibiotics (gentamycin [50 µg/mL], tetracycline [50 µg/mL], ampicillin [50 µg/mL], and penicillin [50 µg/mL]) showed that *Pseudomonas aeruginosa* was susceptible to only three antibiotics (gentamycin, tetracycline, and penicillin) showing a clear zone of inhibition (Fig. 6) while *P. aeruginosa* was resistant to only ampicillin showing no zone of inhibition (Tables 1 and 2).

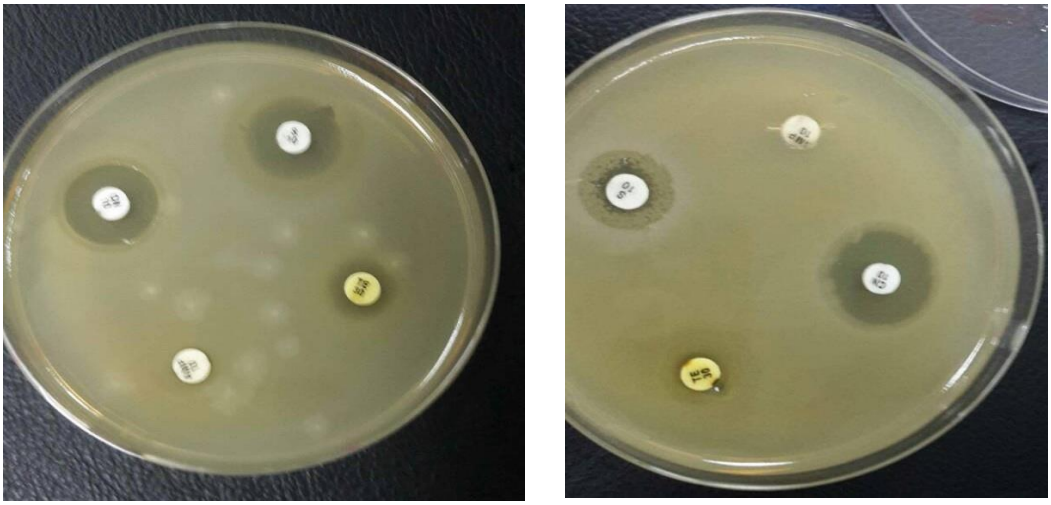

Figure 6. *Pseudomonas* antibiotic disc diffusion test

Table 1: Summary of the antibiotic disk diffusion test

	Gentamycin (50 µg/mL)	Tetracycline (50 µg/mL)	Ampicillin (50 µg/mL)	Penicillin (50 µg/mL)
Pseudomonas aeruginosa	Resistance	Resistance	Susceptible	Resistance

Table 2: Antibiotic sensitivity test for the *Pseudomonas aeruginosa* against different antibiotics using the disc diffusion method

Antibiotics	Replicate 1	Replicate 2	Replicate 3	Mean±SD
Streptomycin	1.5	1.8	1.7	1.67 ± 0.15^{d}
Gentamycin	1	1.4	1.7	1.37 ± 0.35^{c}
Tetracycline	1	1.2	1.1	1.10 ± 0.10^{b}
Ampicillin	0	0	0	0.00 ± 0.00^{a}

The results were analyzed using one-way ANOVA. The values of the various parts with different letters are significantly different ($p<0.05$), as measured by Tukey's HSD post hoc test.

Discussion:

The rapid occurrence of antimicrobial resistance has globally threatened the efficacy of antibiotics, which have not only transformed medicine but also saved several millions of lives. *P. aeruginosa* is a usual cause of Healthcare-Associated Infections involving bloodstream and pneumonia, surgical-site, and urinary tract infections. According to the literature, more than 13% (6,000 of the 51,000) of health care–related to *P. aeruginosa* infections occurring each year are related to multi-drug resistance (MDR) (21). Approximately 400 annual deaths are recognized to *P. aeruginosa* infections with some strains of MDR *P. aeruginosa* being found to be resistant to approximately all antibiotics, including aminoglycosides, carbapenems, cephalosporins, and fluoroquinolones (8 , 22).

P. aeruginosa is of substantial apprehension for cystic fibrosis patients (23); the pathogen is extremely determined and can prevent human immune defenses. Resistance enlargement is related to the extensive antibiotic management of cystic fibrosis patients. The results of antibacterial sensitivity are in agreement with the results of Jombo et al. (24) who also reported resistance to penicillin by the isolates of *P. aeruginosa* while Swetha et al. (25) also reported the resistance of *P. aeruginosa* to ampicillin, penicillin, and oxacillin. Pathogen shows resistance to antimicrobial agents by altering their genome or obtaining approximately acquired resistance mechanism, as a reaction to common abuse and due to the existence of most bacteria in the form of a biofilm containing diverse species that do not only interact with each other but also with their environment (9, 26-29).

Conclusion:

Soil samples are cheap and rich sources for *P. aeruginosa* screening and isolation. The soil isolates are also potential sources for the development of effective antibiotics against resistant bacteria.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Lincoln University.

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العزل والفحص وحساسية المضادات الحيوية لأنواع Pseudomonas من تربة بحيرة كيلانا جايا في سيلانكور ماليزيا

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الخلاصة:

الكائنات الحية الدقيقة المسببة للأمراض من المستشفى والمجتمعات والبيئة تشكل تهديدات كبيرة لصحة الإنسان أصبح تطوير مقاومة المضادات الحيوية مصدر قلق كبير أيضًا. تهدف هذه الدراسة ، بالتالي ، إلى عزل وفحص وتقييم مدى حساسية المضادات الحيوية لأنواع السودوموناس المعزولة ضد أربعة مضادات حيوية (الجنتاميسين والتتر سيكلين والأمبيسيليلين والبنسلين) على لوحة وسائط مولر-هينتون أغار. عدة. تم استخدام نشر القرص وكذلك اختبار الأكسدة من نتيجة الأكسدة الإيجابية ، تم التعرف على البكتيريا المعزولة *مثل Pseudomonas aeruginosa (99.5 – 99.9% ID), Burkholderia cepacia (97.6% ID)، و Pseudomonas fluorescent (75.9% ID).* وقد تبين أن السودوموناس كان عرضة لثلاثة مضادات حيوية فقط (الجنتاميسين ، التتراسيكلين ، والبنسلين) والتي تظهر منطقة تثبيط واضحة بينما كانت الزائفة؟ مقاومة للأمبيسيلين فقط مع عدم وجود منطقة تثبيط. البكتيريا المعزولة هي مصادر محتملة لتطوير المضادات الحيوية الفعالة ضد البكتيريا المقاومة.

الكلمات المفتاحية: المضادات الحيوية، المقاومة البكتيرية، الحساسية ، الزائفة.