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Confirmatory identification and antibiotics resistance profiling of *Pseudomonas aeruginosa*: a laboratory-based study

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ABSTRACT. *Pseudomonas aeruginosa* is a significant opportunistic pathogen frequently found in hospital environments and sewage systems, posing a challenge due to its intrinsic resistance to multiple antibiotics. Five isolates of *Pseudomonas aeruginosa* were collected from the Ahmadu Bello University Teaching Hospital, Shika Zaria and subjected to Gram staining and biochemical tests for initial identification. Four isolates were confirmed to be *Pseudomonas aeruginosa* based on these tests. Antibiotic susceptibility testing was performed to assess their resistance profiles, focusing on Imipenem and Ofloxacin. All four confirmed *Pseudomonas aeruginosa* isolates demonstrated resistance to Imipenem, a carbapenem antibiotic commonly used in clinical settings. Conversely, these isolates showed sensitivity to Ofloxacin, an alternative antibiotic with implications for treatment strategies against *Pseudomonas aeruginosa* infections. The observed resistance to Imipenem underscores ongoing challenges in treating *Pseudomonas aeruginosa* infections, emphasizing the need for alternative therapeutic options such as phage therapy.

Keywords: *Pseudomonas aeruginosa*; antibiotics; resistance; profiling.

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Introduction

Pseudomonas aeruginosa is a prominent member of multi-drug resistance Gram-negative bacteria and is a cause of damaging infections that can be highly resistance to successful treatment with antibiotics (Harper & Enright, 2011). *Pseudomonas aeruginosa* has been described as a "phenomenon of bacterial resistance'' (Strateva & Yodanov, 2009) because it exhibits lots of mechanisms of resistance which include the following: increased expression of efflux system, production of antibiotics degrading or modifying or inactivating enzymes, limited membrane permeability and antibiotics target modification (Bashir & Brown, 2022). In addition to these mechanisms is its growth in dense biofilms, reducing the efficacy of multiple antibiotics (Harper & Enright, 2011). Developing resistance among bacteria towards antibiotics has made the treatment of infections and diseases difficult (Manohar & Ramesh, 2019). Alternative therapy is required to conquer this bacterial resistance to antibiotics. *Pseudomonas aeruginosa* is a ubiquitous, opportunistic pathogen known for causing a range of infections, particularly in immunocompromised individuals (Sathe et al., 2023). It is frequently isolated from hospital environments and is associated with severe infections such as pneumonia, urinary tract infections, and sepsis (Litwin, Rojek, Gozdzik, & Duszynska, 2021). One of the most concerning attributes of *Pseudomonas aeruginosa* is its intrinsic resistance to many antibiotics and its ability to acquire additional resistance mechanisms, making infections difficult to treat and leading to high morbidity and mortality rates. Accurate identification of *Pseudomonas aeruginosa* is critical for effective clinical management and infection control. Misidentification can result in inappropriate antibiotic therapy, further complicating treatment outcomes. Traditional biochemical methods, while useful, sometimes lack the sensitivity and specificity required for definitive identification. Molecular techniques, although more precise, may not be routinely available in all laboratory settings. In addition to identification, antibiotic resistance profiling of *Pseudomonas aeruginosa* is essential for guiding appropriate antimicrobial therapy. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has exacerbated the challenge of treating infections caused by this pathogen. Understanding the resistance patterns of *Pseudomonas aeruginosa* isolates can inform treatment decisions and help in the development of effective antibiotic stewardship programs. This study aims to reconfirm the identification of collected *Pseudomonas aeruginosa* isolates and conduct comprehensive antibiotic sensitivity testing. By combining traditional and

advanced identification methods with detailed resistance profiling, this research seeks to provide valuable insights into the accurate identification and effective treatment of *Pseudononas aeruginosa* infections.

Pseudomonas aeruginosa

Pseudomonas aeruginosa was first described as a different bacterial species at the end of the nineteenth century, after a sterile culture media was developed by Pasteur. In 1882, the first scientific study on *Pseudomonas aeruginosa* entitled "On the blue and green colouration of bandages," was published by a pharmacist named Carle Gessard. *Pseudomonas aeruginosa* is a gram-negative, aerobic, non-spore-forming rod that can cause a wide variety of human infections (Wilson & Pandey, 2022).

Pseudomonas aeruginosa is often isolated from plants, fruits, soil and water environments such as Lakes, rivers and swimming pools. In particular circumstances, *Pseudomonas aeruginosa* is an important pathogenic factor of severe and often opportunistic infections in humans. *Pseudomonas aeruginosa* typically infects airways and urinary tracts, it also a causative agent of few blood blood infections, wound /burn injury infections, hot-tub dermatitis and outer ear infections (swimmers ear), joint and bone, soft tissue, bacteremia are more common in patients with tuberculosis, burns, cancer and AIDS (Wu & Li, 2015). The natural and acquired resistance of *Pseudomonas aeruginosa* to antibiotics can result to difficulty in treating the infection or disease caused by this organism (Breidenstein, de la Fuente-Núñez, & Hancock, 2011). *Pseudomonas aeruginosa* demonstrate three types of colonies which are; natural isolates from the soil and water which typically appear as small, rough colony, while clinical isolates are likely smooth colony types, occasionally with fried egg appearance that is flat, smooth, with flat edges and elevated appearance. While the urinary and respiratory isolates may present a mucoid-type (alginate slime) appearance (Sapkota, Manandhar, & Aryal, 2023).

Morphology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a non-spore forming, non-capsulated bacterium (but some strains possess slime layer), rod shaped (Bacillus), slender, gram negative bacterium, with size ranging from about 1.5 - 3 µm. The cells of *Pseudomonas aeruginosa* are arranged singly or in pairs. They are actively mortile, and motility is my means of polar flagellum arranged in an amphitrichous manner (Batra, 2018).

Multi-drug resistance *Pseudomonas aeruginosa*

Pseudomonas aeruginosa are gram negative nosocomial pathogens that is known for causing variety of diseases usually in wound and respiratory tract etc. (Meletis & Bagkeri, 2013). The *Pseudomonas aeruginosa* genome is one of the biggest in the bacterial entity supporting its great genetic capacity and high adaptability to changes in the environment. Antibiotics resistance mechanism of *Pseudomonas aeruginosa* are divided into two which intrinsic and extrinsic resistance.

The intrinsic resistance is refers to resistance which result from large selection of genetically-encoded mechanism. While the acquired resistance is as a result of gaining an additional mechanism or mutation under selective pressure (Meletis & Bagkeri, 2013).

Clinical manifestation of the disease caused by *Pseudomonas aeruginosa*

Pseudomonas is a group of bacteria that can cause different infections, and most of the diseases are often caused by the specie *Pseudomonas aeruginosa*, which primarily is nosocomial but can also be transmitted from hot tubs and swimming pools (Bennington-Castro, 2015). Symptoms of *Pseudomonas aeruginosa* infection vary based on the organ affected.

Lungs (pneumonia): resulting in fever, chills, difficulty breathing, chest pain, tiredness, cough sometimes with yellow, green or bloody mucus.

Urinary tract infection: with symptoms such as frequent urination, dysuria, cloudy or bloody urine, pain in the pelvic area etc.

Wound infection: which can cause inflammation at the wound site and also pus from the wound.

Swimmer's ear (external otitis): This is a mild external infection that can occur in both healthy and immune-suppressed causing ear pain, decreased hearing, discharge from the ear, erythema in the outer part of the ear and lastly fever (Bennington-Castro, 2015)

Bloodstream infection: which often occur when the bacteria enters the bloodstream either through injection of contaminated illegal drug into the vein, intravascular catheter etc.

Risks factors

In hospital, *Pseudomonas aeruginosa* is spread through improper hygiene such as unclean hands of the health workers or via the use of contaminated medical equipment that are not properly sterilized. Patients in the hospital are also at higher risk of acquiring the infection if unsterilized surgical materials are used on wounds or burns, or are being treated with breathing machines such as the mechanical ventilator or other medical appliances such catheters. Swimmers are also at high risk of acquiring the infection through unfrequently disinfected swimming pools or hot tubes and contact lens etc. (Bennington-Castro, 2015).

Laboratory diagnosis

Blood, sputum or other body fluid can be examined using culture for the presence of the organism and also antibiotics susceptibility test can be done to determine the suitable antibiotics against the organism (Bennington-Castro, 2015)

Treatment

Antibiotics are applied topically, orally or given intravenously depending on the site of the infection. Swimmer's ear can be treated effectively by irrigating the ears with acetic acid (vinegar) solution before and after swimming and polymyxin can also be use in treating the infection by topical application on the ears.

Urinary tract infection can be treated with levofloxacin or Ciprofloxacin orally. Serious infection due to *Pseudomonas aeruginosa* (Pneumonia and blood infection) requires weeks of antibiotics given intravenously such as ceftazidime or Ciprofloxacin are effective. In most cases, combination of antibiotics is usually required because many strains, particularly nosocomial acquired infections are resistance to many antibiotics and are difficult to treat.

Prevention and control

Frequent washing of hands after use;
Avoid sharing of personal items;
Disinfection of surfaces such a cell phones, door knobs, and laboratory benches;
Frequent chlorination of swimming pools and hot tubs;
Hospital devices should sterilize after each and every use.

Material and methods

All the media were prepared according to manufacturer's instruction and were sterilized in the autoclave at 121 ºC for 15 minutes. Five (5) plates of well identified and characterized isolates of *Pseudomonas aeruginosa* were collected from Ahmadu Bello University Teaching Hospital, Shikka Zaria. The isolates were sub-cultured on cetrimide agar and the plates were incubated at 37 °C for 24 hours. After which growth were observed on all the plates. Discrete colony of the bacteria from the incubated plate was transferred to a grease free slide containing a loop full of water to make a smear, the smear was heat fixed by passing the slide over a flame three times, the slide was flushed with crystal violet, iodine, alcohol and safranin, and was washed after allowing it for 60 seconds (with the exception of the decolorizer which was washed after 5 seconds) respectively. The stained slide was air-dried and viewed under the microscope at × 100 oil immersion. Four biochemical tests were carried out. These biochemical tests include catalase test, oxidase test, Oxidative-fermentative test and lastly citrate utilizing test. 0.28 g of citrate was dissolved in 100 mL of water, the mixture was boiled to dissolve the precipitate and was autoclaved at 121°C for 15 minutes. After autoclaving, the citrate was dispensed into 5 universal bottles and was allowed to solidify in a slanted position. A colony from the isolate was picked using a sterile straight wire and was inoculated into the slanted mixture by stabbing the bud and streaking the surface. The bottles were incubated at 18 – 37 °C for 24 hours. One drop of 3% H2O2 was dropped on a clean grease free glass slide. A colony from the isolate was picked using a sterile tooth pick and was emulsified with the H_2O_2, the slide was observed after 10 seconds for bubble formation. The test was done by putting a drop of oxidase reagent (Tetra-methyl-p-phenylenediaminedihydrochloride) and a colony of the isolate on a clean filter paper simultaneously, the mixture was emulsified and was left for 10 - 15 seconds for color change. 1.5 g of O.F basal medium was suspended into 150 mL of distilled water. The mixture was then heated to boiling to dissolve the medium completely, the solution was then dispensed in 5 mL amount and sterilized by autoclave at 15 lbs pressure (121 ºC) for 15 minutes. To first 5 mL of sterile basal medium, 0.5 mL sterile 10% dextrose solution was added to second 5 mL, 0.5 mL sterile 10% lactose was added.

To third 5 mL, 0.5 mL sterile 10% saaccharose solution was added. The solutions were mixed and aseptically dispensed into sterile test tubes in duplicate for aerobic and anaerobic fermentation. The agar used for the anti-microbial susceptibility test was Mueller-Hinton agar, and the test were done using the Kirby-Bauer disk diffusion. A colony of *Pseudomonas aeruginosa* was picked from the culture plate and was inoculated into normal saline and standardised using the 0.5 MacFaland standard. The surface of the Muller-Hinton agar plate was inoculated by spreading the swab over the entire sterile agar plate. The following antibiotics used are Imipenem (10 µg), Piperacillin-tazobactam (100 10 μg^{-1}), Amikacin (30 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), and Oflaxacin (10 µg). The antibiotics discs were placed aseptically on the agar 6 mm away from each other. The plates were allowed for 30 minutes for the antibiotics disc to diffuse and were incubated at 37 °C for 16 – 18 hours.

Results

On culturing the clinical isolates of *Pseudomonas aeruginosa*, the colonies appeared as blue-greenish colony (Pyocyanin), smooth, flat edges with grape-like ordor on cetrimide agar. The Gram reaction of the stained colony was observed reddish-pink bacilli. The biochemical characteristics of the presumptive *Pseudomonas aeruginosa* following isolation on cetrimide agar where colonies were positive to catalase, oxidase, and citrate utilization but negative to sugar fermentation test. The antibiotics susceptibility pattern of *Pseudomonas aeruginosa* where the susceptible antibiotics are piperacillin-Tazobactam (50%), Amikacin (50%), Ciprofloxacin (75%), Gentamycin (50%), and Oflaxacin (100%), the antibiotics with intermediate resistance are Tazobactam (50%), Amikacin (50%), Ciprofloxacin (25%), and Gentamycin (25%). Lastly the resisted antibiotics are Gentamycin (25%) and imipenem (100%) as shown in Tables 1 and 2.

Table 1. The Microscopic and Biochemical characterization of clinical isolates of *Pseudomonas aeruginosa*.

Isolate number	Gran reaction	Oxidase test	Catalase test	Citrate utilization test	Oxidative fermentative	Inference
IS1	-rod	+	+	+	Oxidative	*P. aeruginosa*
IS2	-rod	+	+	+	Oxidative	*P. aeruginosa*
IS3	-rod	+	+	+	Oxidative	*P. aeruginosa*
IS4	-rod	+	+	+	Non Oxidative	-
IS5	-rod	+	+	+	Non Oxidative	-

Keys: IS = isolate; + = positive; - = negative.

Table 2. The antibiotics susceptibility pattern of *Pseudomonas aeruginosa* isolates from ABUMC.

Antibiotics (Disc potency µg)	No of resistant (%)	No intermetiate (%)	No sensitive (%)
Piperacilin Tazobactan (100/10)	0 (0%)	2 (50%)	2 (50%)
Imipenem	4 (100%)	0 (0%)	0 (0%)
Amikacin	0 (0%)	2 (50%)	2 (50%)
Ciprofloxacin	0 (0%)	1 (25%)	3 (75%)
Gentamycin	1 (25%)	1 (25%)	1 (25%)
Oflaxacin	0 (0%)	0 (0%)	4 (100%)

Discussion

From the collected isolates, 80% were confirmed as *Pseudomonas aeruginosa* which implies that the method of isolation and characterization employed in the hospital is effective and reliable. From this study, the most ineffective antibiotic for the treatment of *Pseudomonas aeruginosa* was imipenem, which is in contrast with the previous study (Olalekan et al., 2022) who studied high incidence of carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from Lagos, Nigeria and reported 39% as rate of resistance to imipenem. The higher resistance of *Pseudomonas aeruginosa* in our study may be associated with possible increase in environmental resistance agents like plasmids or maybe due to antibiotics abuse. Mutation in the outer membrane D (OPrD) might have also caused resistance of the *Pseudomonas aeruginosa* to imipenem, were deficiency of the outer membrane protein D (OprD) gives *Pseudomonas aeruginosa* a basal level of resistance to carbapenems, especially to imipenem, were studies has revealed that loops 2 and 3 in the OprD protein contain the entrance and/or binding sites for imipenem. Therefore, any mutation in loop 2 and/or loop 3 that causes conformational changes could result in carbapenem resistance as described by (Li, Luo, Williams, Blackwell, & Xie, 2012).

Conclusion

In conclusion, the study confirms that the methods for identifying *Pseudomonas aeruginosa* in clinical samples are reliable, with an 80% success rate. The tests also showed important results regarding how these bacteria respond to antibiotics. It was found that all tested strains were resistant to imipenem, a powerful antibiotic often used against these bacteria. This resistance is concerning and highlights the need for careful use of antibiotics to prevent further resistance. On the other hand, all strains were sensitive to ofloxacin, which means this antibiotic could be a good choice for treating infections caused by *Pseudomonas aeruginosa*. The findings provide valuable information for doctors choosing antibiotics and for developing strategies to control infections in hospitals. Future research should focus on understanding why these bacteria are becoming resistant and finding new treatments. In summary, the study not only shows the methods work well for identifying *Pseudomonas aeruginosa* but also gives important information about which antibiotics might be most effective against these bacteria.

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