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Identification and Antimicrobial Susceptibility of *Pseudomonas aeruginosa* strain PAO1 Isolated from African Catfish Skin Scrapings

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Abstract

The current emergence of multidrug-resistant bacteria has posed a significant challenge to public health in Nigeria and Africa as a whole, necessitating studies to evaluate the effectiveness of antibiotics and understand bacterial resistance mechanisms. This study aimed to isolate bacteria from African catfish skin using the spread plate technique and assess antibiotic susceptibility patterns through the diffusion method. The antibiogram results revealed that tarivid exhibited the highest antibacterial activity against a newly isolated *Pseudomonas aeruginosa* strain PAO1 (96%), followed by ciprofloxacin (78%), chloramphenicol (63%), sparfloxacin (58%), and rufloxacin (56%). However, *P. aeruginosa* PAO1 demonstrated strong resistance to gentamycin (81%), streptomycin (100%), cloxacillin (100%), cefalexin (100%) and cotrimoxazole (100%). To further elucidate the resistance mechanisms, the findings indicate that while *P. aeruginosa* infections are highly sensitive to tarivid and ciprofloxacin, their use does not guarantee eradication of all bacterial strains due to the organism's multidrug-resistant nature. Improper administration of these drugs could further contribute to resistance development. This study underscores the importance of targeted antibiotic therapy and the need for careful monitoring of drug use to mitigate the spread of resistant bacterial strains.

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Introduction

Pseudomonas aeruginosa is an aerobic, unipolar, Gram-negative coccobacillus bacterium, an opportunistic human and plant pathogen [1]. *P. aeruginosa* is the type species of the genus *Pseudomonas*. Pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown) are among the pigments secreted by *P. aeruginosa*. Pseudomonas Agar P (King A medium) was created by King, Ward and Raney to increase the synthesis of pyocyanin and pyorubin, and Pseudomonas Agar F (King B medium) to increase the production of fluorescein [2]. The pearlescent appearance and grape-like or tortilla-like odor of *P. aeruginosa* are frequently used as preliminary identification methods *in vitro*. The ability to grow at 42°C and the generation of both pyocyanin and fluorescein are frequently necessary for definitive clinical identification of *P. aeruginosa* [1].

An opportunistic pathogen that is common in the environment is *P. aeruginosa*. When *P. aeruginosa* penetrates a human host's initial line of defenses and enters the body through the skin at the location of an open wound, epidermal infections frequently ensue. *P. aeruginosa* is frequently found in hospital bacterial populations, where it can infect burn patients and other vulnerable people. The likelihood that *P. aeruginosa* will enter the circulation through the burn victim's exposed deep epidermal tissue after severe skin damage is increased by the organism's environmental prevalence [3]. *P. aeruginosa* is an opportunistic nosocomial pathogen that is responsible for causing urinary tract infections, respiratory system infections, dermatitis, otitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed [1]. According to prevalence surveys, *P. aeruginosa* accounts for between 27.78% to 33.3% of the bacteria detected in wounds and is the most common Gram-negative organism recovered from wound infections, usually, with wounds from surgery [4, 5]. According to prevalence studies, *P. aeruginosa* infections are more common in patients between the ages of 21 and 40. Male patients are more likely to have *P. aeruginosa* infections (58%) compared to 42% in female patients [6]. With a 50% mortality rate, *P. aeruginosa* is the most common pathogen that causes nosocomial infections-infections that patients acquire while receiving treatment for other conditions, for example, in patients with compromised host defense mechanisms [6]. These infections are the most severe and life-threatening infections, especially in hospitalized patients with serious underlying diseases, in intensive care units, in patients with severe burns, in cancer patients and immunocompromised patients undergoing transplantation [7, 8]. A combination of compromised human defenses, antibiotic-resistant bacteria and the generation of extracellular bacterial enzymes and toxins contribute to the high fatality rate linked to these illnesses adding to its pathogenicity [9]. *P. aeruginosa* has minimal nutritional requirements and can tolerate a wide variety of physical conditions, it can adapt in many ecological niches, from water and soil to plant and animal tissues [10]. Because they can use a variety of organic substances as food, the bacteria have a remarkable capacity to colonize ecological niches with low nutrition availability [11].

Pneumonia, bloodstream infections, urinary tract infections and surgical site infections are among the main healthcare-associated infections caused by *P. aeruginosa* [12]. Out of 51,000 *P. aeruginosa* infections linked to healthcare in the United States annually, over 6,000 (13%) are multidrug resistant. It is estimated that these diseases cause about 400 fatalities annually [12]. It has been discovered that *P. aeruginosa* is resistant to almost all antibiotics, including carbapenems, aminoglycosides, cephalosporins, and fluoroquinolones [12, 13]. Isolation of *P. aeruginosa* from *Clarias gariepinus* is significant due to its implications for aquaculture practices, public health, and food safety. As a multidrug-resistant pathogen, its presence in a widely consumed fish species in Nigeria raises concerns about zoonotic transmission, antibiotic resistance and potential economic losses from fish mortality and treatment costs. Additionally, it highlights the need for improved water quality, hygiene and sustainable farming practices to prevent environmental contamination and safeguard animal and human health. Monitoring and addressing this pathogen are essential for ensuring safe aquaculture and mitigating resistance risks.

Materials and Methods

Study area

This research work was carried out in Gwagwalada Area Council Abuja, FCT, North-Central Zone of Nigeria. The town has a land mass of 7, 315 sq km, of which 1, 043 sq km are occupied by Gwagwalada. It is situated at latitude 7 ºE and longitude 8 ºN. It is

located in the Savannah region, which has a temperate climate [14].

Isolation and identification of *Pseudomonas aeruginosa*

The isolation of bacteria was done using the spread plate technique described [1]. Samples were collected from the skin of 120 carefully selected *Clarias gariepinus* Catfish. A total of 120 sterile swab sticks were used to collect samples from the skin of the fish, forty swabs from each pond. The three different ponds are: Pond A, (Andrew Azazi Barack Earthen Pond); Pond B, (Muhammad Salihu's Concrete Pond phase 3) and Pond C, (Abdullahi's Earthen Pond Dagiri). Some drops of normal saline were introduced into the swab sticks, the swab sticks were spread on an already solidified MacConckey agar, Cetrimide agar and Cled agar. The plates were allowed to stay undisturbed for 15 minutes. Then, the plates were inverted and incubated at 37℃ for 24 hours. Later, the plates were examined and discrete colonies were picked with a sterile wire loop and streaked on nutrient agar. The plates were inverted and incubated for another 24 hours. The plates were observed for growth and colonial morphology such as; size, shape, optical characteristics, elevation and color change. The presence of blue-green coloration on a cetrimide agar, CLED agar and nutrient agar signifies the presence of *P. aeruginosa* named PAO1. *P. aeruginosa* PAO1 was identified by using conventional biochemical tests and the Api system (Biomeraux, France) [1].

Antibiogram of *Pseudomonas aeruginosa*

The inoculum was prepared using 18 hours of culture. A sterile swab stick was used to pick the 18-hour culture onto the normal saline until the turbidity was equivalent to 0.5 MacFarland standard. *P. aeruginosa* PAO1 inoculum was seeded onto the surface of Mueller–Hinton agar plates and allowed for a few minutes at room temperature. Antibiogram was performed using antibiotic discs such as; amoxiclav 10 µg, cefalexin 10 µg, clindamycin 2 µg, cloxacillin 1µg, cotrimoxazole 25 µg, erythromycin 15 µg, tetracycline 30 µg, septrin 30 µg, chloramphenicol 30 µg, sparfloxacin 10 µg, gentamycin 10 µg, rufloxacin 30 µg, tarivid 10 µg, streptomycin 30 µg, ciprofloxacin 10 µg on the surface of Mueller-Hinton agar, using the standard disc diffusion method recommended by the National committee for clinical laboratory standards. This was done by dipping a sterile swab stick into normal saline containing the culture organism and carefully swabbing the entire surface of Mueller–Hinton agar plates and allow to stay for a few minutes. The antibiotic discs (6 mm) were impregnated carefully onto the seeded organisms on Mueller-Hinton agar and then plates were incubated for 24 hours at 37°C. The zone layers of inhibition were observed and recorded.

Molecular characterization of *P. aeruginosa* PAO1

This involves a series of examinations and analyses on a DNA strand such as thermal lysis, denaturation, annealing of primer, and extension of DNA strands, followed by amplification and finally purifications for proper imaging. The DNA extraction buffer (1 mM EDTA, 1 M NaCl, and 2% (w/v) PVP, 1% (v/v) β-mercaptoethanol, pH 8.0) was added just before use, while the TE Buffer at pH 8.0 contains 100 mM Tris and 10 mM EDTA were used.

Genomic DNA extraction and quality determination

The DNA extraction began by scraping a single colony from the agar culture into a sterile Eppendorf tube using a sterile pipette tip. The cells were added in 567 µl of TE buffer and suspended either by pipetting the pellet several times or swirling a toothpick in the solution. Next, 3 µl of proteinase K solution (20 mg/mL) and 30 µl of 10% SDS were added to the tube and the mixture was incubated at 37°C for 1 hour. Following incubation, 100 µl of 5M NaCl was mixed into the solution, followed by the addition of 80 µl of CTAB/NaCl solution (0.7M NaCl, 10% CTAB). The mixture was then incubated at 65°C for 10 minutes. Afterward, 750 µl of phenol was added and mixed with an equivalent volume of chloroform:isoamyl alcohol (24:1). The solution was centrifuged at 12,000 rpm for 10 minutes and 600 µl of the supernatant was transferred to a fresh tube. To precipitate the DNA, 0.6 volumes (360 µl) of isopropanol were added and the mixture was incubated at -20°C for 30 minutes. The precipitated DNA was centrifuged and the isopropanol was discarded. The pellet was washed with 500 µl of 70% ethanol to remove residual salts, centrifuged again, and the ethanol was discarded. The DNA was dried at 37°C for 30 minutes, resuspended in 50 µl of sterile distilled water and stored at -20°C. The quality of the DNA was checked on a 1.5% agarose gel. Where 4µl of the stock DNA was mixed with 4 µl loading dye and ran at a voltage of 120V for 45 min. This was later viewed under UV light to confirm the presence and quality of the bacteria's DNA. High molecular band size indicates the presence of DNA in each sample loaded in the wells. Also, the quality and quantity

assessment of extracted DNA was carried out using Nanodrop Spectrophotometer 2000. One µl of stock DNA was pipetted into the sensor of the Nanodrop and readings were taken to determine the quantity in concentration (ng/ µl) of the extracted DNA and also determine purity by measuring at 260/280 nm amounts of proteins and at 260/230, the amount of alcohol residue left in the DNA extracts.

Polymerase chain reaction (PCR)

Ten µl of 5× GoTaq colorless reaction buffer, 3 µl of $MgCl_2$, 1 µl of 10 mM dNTPs mix, 1 µl of 10 mM forward (27 F) and reverse primers (1492 R), and 0.3 units of Taq DNA polymerase (Promega, USA) made up the PCR reaction cocktail, which was built up to 42 µl with sterile distilled water and 8 µl of diluted DNA template. A GeneAmp 9700 PCR System heat (thermal) cycler (Applied Biosystem Inc., USA) was used for the PCR. A cycle of initial denaturation at 94°C for 5 minutes was followed by 35 cycles of each cycle, which consists of 30 seconds of denaturation at 94°C, 30 seconds of primer annealing at 50°C, 1.5 minutes of extension at 720°C and a final 7-minute extension at 72°C. Note that the working DNA solution was diluted at 1:50 for subsequent PCR assay. The amplified fragment was checked on a 2% agarose gel run on a voltage of 120V for 45 min. This was later viewed under UV light to confirm the presence of the amplified PCR products.

Purification of amplified PCR product

The PCR reagents were eliminated by ethanol purification of the generated fragments. Each 50 µl PCR amplified product was mixed with 2.5 volume (125 µl) of 95% ethanol in a fresh, sterile 1.5 µl tube Eppendorf. The mixture was then vortexed or simply inverted, and kept at -20°C for at least 30 minutes or one hour. After 10 minutes of centrifugation at 12000 g and the removal of the supernatant, the pellets were cleaned by adding 500 µl of 70% ethanol, mixing and centrifuging for 5 minutes at 12000 g. Removed all of the supernatant once again, repeated the washing process and then placed it in the incubator at 37°C for 25 to 30 minutes to dry. The amplified PCR product was re-suspended in 30 µl of sterile distilled water and stored in a freezer at -20°C. The existence of the target gene was confirmed by testing the purified fragment on a 2% agarose gel that was operated at 120V for approximately 45 minutes. The sequencing of the 16S rRNA amplified gene was performed at the 176 Guards Battalion Brigade Laboratory, Gwagwalada Abuja, Nigeria. The sequence of isolated strain was Blast identified as *P. aeruginosa* and submitted to NCBI. The accession number NC002516.2 was allotted to *P. aeruginosa* POA1.

Results and Discussions

Morphological characteristics of *P. aeruginosa* PAO1 on different media

Morphological characteristics of *P. aeruginosa* PAO1 isolated from fish scrapings on different agar media were carried out based on the colony shape, size, elevation, edge, optical characteristics, consistency, surface, pigmentation and odor as shown in Table 1. The morphological features of *P. aeruginosa* PAO1 on different media, such as MacConkey agar, nutrient agar and CLED agar were very similar, even though *P. aeruginosa* PAO1 produces circular, mucoid, smooth colonies with emit of sweet grape odor in all the agars. *P. aeruginosa* PAO1 grew well on MacConkey agar but did not ferment lactose sugar. *P. aeruginosa* PAO1 did not produce any characteristics pigment either on nutrient agar or on MacConkey agar, which was observed to be due to low-temperature levels (*i.e.*, temperature below 37°C) and some atypical strains of *P. aeruginosa* strains that do not produce pigments on

Table 1 Morphological characteristics of *Pseudomonas aeruginosa* PAO1 on different agar media.

Morphological features	Mac agar	NA agar	CLED agar
Colony shape	Circular	Circular	Irregular
Colony size	Large	Medium	Medium
Colony elevation	Raised	Convex	Raised
Colony edge	Entire	Entire	Undulate
Optical characteristics	Opaque	Opaque	Opaque
Consistency	Mucoid	Mucoid	Mucoid
Colony surface	Smooth	Smooth	Rough
Colony pigmentation	Colorless	Blue-green	Blue-green
Colony odor	Sweet grape	Sweet grape	Sweet grape

Mac agar = MacConkey agar, NA agar = Nutrient agar, CLED agar = Cysteine, lactose and electrolyte-deficient agar

Table 2 Biochemical characteristics of *Pseudomonas aeruginosa* PAO1.

Biochemical tests	Results
Gram staining	Negative
Motility test	Positive
Catalase	Positive
Oxidase	Positive
Citrate	Positive
Indole	Negative
Methyl red	Negative
Voges-Proskauer	Negative

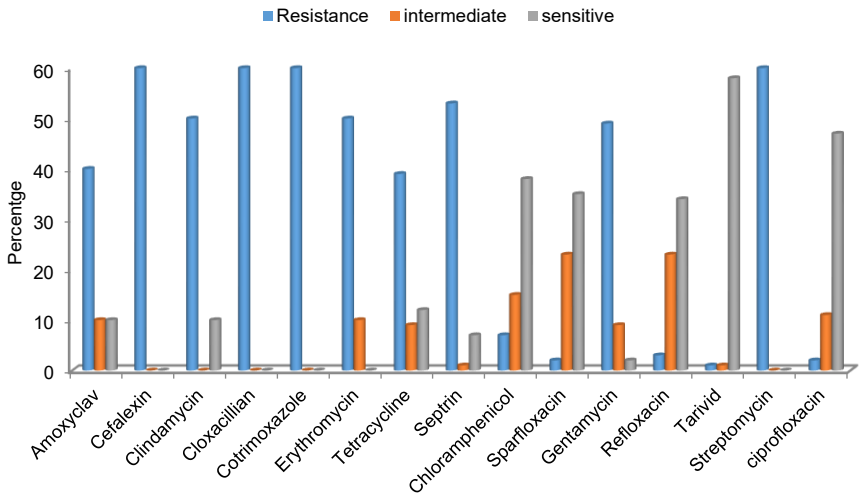

Fig. 1 Antibiogram of *Pseudomonas aeruginosa* PAO1 from fish scrapings showing resistance, intermediate and sensitive effect on *P. aeruginosa* PAO1 for each antibiotic.

agar media have been reported earlier [1]. Many strains of *P. aeruginosa* PAO1 produce various types of pyocins and this pyocin-producing strain of *P. aeruginosa* PAO1 gives pigment on agar media.

Biochemical characteristics of *Pseudomonas aeruginosa* PAO1

Table 2 shows the biochemical test results of *P. aeruginosa* PAO1. As an autochthonous member of an aquatic and soil habitat, *P. aeruginosa* PAO1 is a motile, asporogenous, non-lactose fermenter bacteria show a positive reaction towards catalase, oxidase and citrate test, while most of the other biochemical test (indole, methyl red, sugar fermentation test, and Voges-Proskauer) were negative. These observations were similar to the reported characteristics of *P. aeruginosa* strains [1].

Antibiogram of *Pseudomonas aeruginosa* PAO1

The susceptibility of *P. aeruginosa* PAO1 to different antibiotics is shown in Fig. 1. The susceptibility of P. aeruginosa PAO1 varies to different antibiotics depending on their susceptibility and resistance patterns [17]. From this research work, it can be concluded that the infection caused by *P. aeruginosa* can be treated with tarivid, ciprofloxacin and chloramphenicol. On the other hand, PAO1 was highly resistant to cefalexin, cotrimoxazole and cloxacillin. Antibiogram tests also known as sensitivity tests were conducted in lab settings or *in vitro* to determine how sensitive antibiotics are to particular bacteria that cause particular diseases. In contrast, this test is used to determine how well antibiotics work to stop bacterial growth *in vitro*. Disc diffusion (Kirby-Bauer method) was used to examine *P. aeruginosa* PAO1 susceptibility and resistance. Of all the antibiotics that were evaluated, streptomycin, cloxacillin, cefalexin, and cotrimoxazole showed the

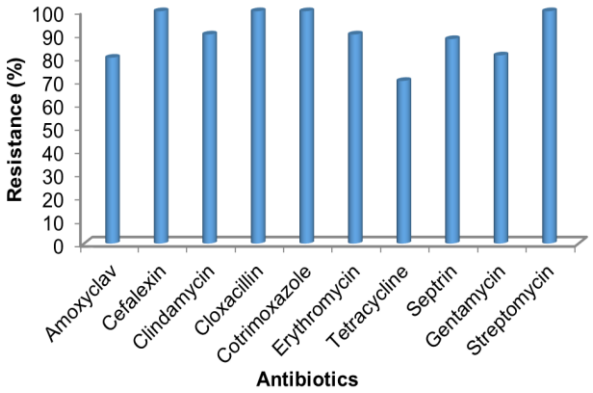

Fig. 2 Antibiogram of *Pseudomonas aeruginosa* PAO1 resistance to antibiotics.

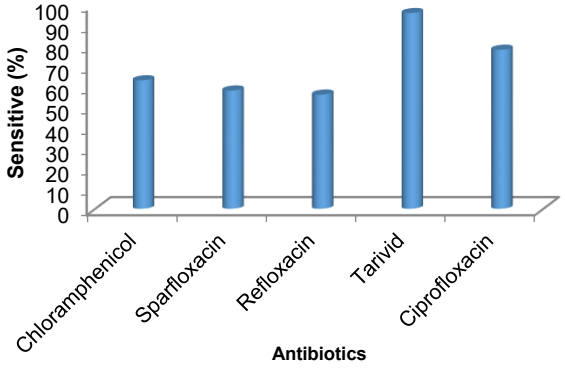

Fig. 3 Antibiogram of effective antibiotics against *Pseudomonas aeruginosa* PAO1.

lowest levels of suppression (Fig. 2). This finding is in contrast with the previous work [19, 20] where the researchers concluded that 100% of *P. aeruginosa* strains were resistant to augmentin, streptomycin, cloxacillin, cefalexin and cotrimoxazole. On the other hand, some antibiotics had intermediate effects, *e.g.*, sparfloxacin, rufloxacin and gentamycin. These results are nearly comparable with some previous reports [21, 24], where *P. aeruginosa* was moderately affected by different antibiotics including gentamycin. The highest sensitivity of *P. aeruginosa* PAO1 was found against tarivid, ciprofloxacin and chloramphenicol (Fig. 3). These results of antibiotic sensitivity were similar to those of previous findings [11, 19, 22, 24], where the authors concluded that *P. aeruginosa* is highly susceptible to ciprofloxacin and tarivid. The rising epidemic of antimicrobial resistance and the subsequent restrictions on antibiotic use in many countries have emphasized the need for effective antimicrobial testing to identify suitable treatment options and mitigate the risks associated with resistant pathogens [19, 23].

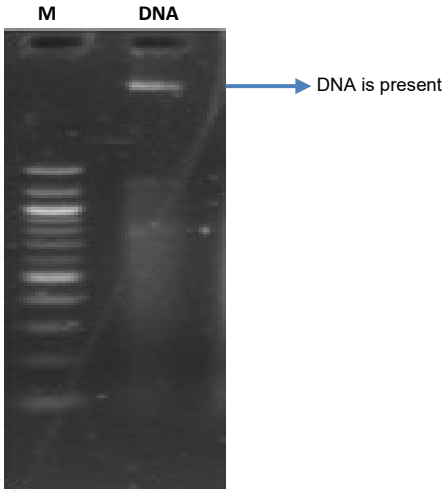

Fig. 4 *Pseudomonas aeruginosa* PAO1 DNA viewed under UV.

Quality assessment of extracted DNA and amplified PCR product by agarose gel electrophoresis

The quality of the DNA was checked on a 1.5% agarose gel. Where 4ul of the stock DNA was mixed with 4 µl loading dye and ran at a voltage of 120V for 45 min. This was later viewed under UV light to confirm the presence and quality of the bacteria DNA. High molecular band size indicates the presence of DNA in sample loaded in the wells. The DNA quantity was found 2374 ng/µl with 26/280 ratio of 2.12 and 260/230 ratio of 2.34 (Fig. 4). Similarly, a 1500 bp band of 16S rRNA was visible after agarose gel electrophoresis PCR amplification (Fig. 5).

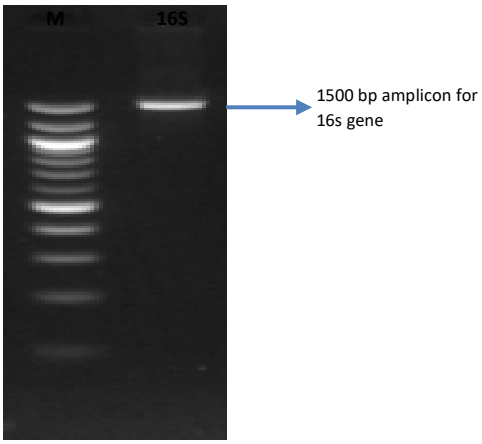

Fig. 5 Amplified 16S rRNA gene of *Pseudomonas aeruginosa* PAO1.

Conflict of Interest

The authors have no conflicts of interest.

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