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Assessing the Impact of Antibiotics on Resistant Strains of *Pseudomonas aeruginosa*

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ABSTRACT:

Pseudomonas aeruginosa is a Gram-negative opportunistic bacillus capable of causing severe infections, such as ventilator-associated pneumonia, sepsis, urinary tract infections, gastrointestinal tract infections, skin infections, and bone-joint infections. *P. aeruginosa* rapidly develops antibiotic resistance during infection and treatment, making it a significant clinical challenge.

Twenty-eight isolates of suspected *P. aeruginosa* were obtained from clinical specimens, and standard biochemical and culture techniques were used to identify the isolates. The VITEK 2 compact system was used for identification and antibiotic susceptibility tests; further confirmation of isolates was achieved using the 16S rRNA gene. Growth curves were generated using a 96-well microtiter plate for four selected strains under normal and antibiotic stress conditions.

The VITEK 2 compact system and molecular diagnostic confirmation showed that 20 isolates were *P. aeruginosa*. Four isolates were selected to represent varying resistance profiles: A2 (susceptible control), A3 and A4 (resistant to all tested antibiotics), and A9 (resistant to three antibiotics, Piperacillin/tazobactam, Ceftazidime and Cefepime). Growth curve analysis demonstrated a steady increase in A2 over time, statistical comparisons were performed One way ANOVA and Tukey's test. Wherein A9 exhibited the most significant growth inhibition ($p < 0.001$), followed by A4 and A3 ($p < 0.001$) and ($p= 0.016$) respectively. Suggesting a potential antibiotic synergy effect.

In conclusion, this study underscores the resilience of multidrug-resistant *P. aeruginosa* strains, which exhibited the ability to resist despite repeated exposure to a diverse array of antibiotics. These findings highlight the urgent need for further molecular investigations into the mechanisms of resistance.

Keywords: *Pseudomonas aeruginosa*, MDR, XDR, Antibiotics, Growth curve

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1 INTRODUCTION

Pseudomonas aeruginosa, a member of the Pseudomonadaceae family, is an opportunistic Gram-negative bacillus. *P. aeruginosa* is present in various habitats, including soil and water, as well as living organisms such as plants and animals. It has the potential to result in severe infections that can be life-threatening, including ventilator-associated pneumonia, sepsis, urinary tract infections, gastrointestinal tract infections, skin infections, and bone-joint infections [1].

P. aeruginosa has the capability to develop antibiotic resistance during infection and antibiotic therapy. The Infectious Diseases Society of America has categorized *P. aeruginosa* as one of the "ESKAPE" (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp.) organisms that are associated with antimicrobial resistance (AMR) and worldwide health threads [2, 3]. The lack of proper infection control measures and improper use of antibiotics is known to accelerate the rate of antibiotic resistance in Pseudomonas spp. This acquired resistance to antimicrobial agents limits available treatment choices and complicates the management of illnesses [4]. Antimicrobial resistance in *P. aeruginosa* can be classified into two categories: inherent and acquired resistance. Intrinsic resistance includes many factors, such as the low permeability of the outer membrane, the presence of efflux pumps, and the creation of enzymes that prevent antibiotics from working. Horizontal gene transfer and chromosomal gene modifications result in the acquisition of acquired resistance [5, 6].

P. aeruginosa infections are becoming increasingly challenging to manage due to the inherent resistance of this bacterium to numerous antibiotics and the global rise in the number of strains that are resistant to multiple and all available drugs. Although the incidence of *P. aeruginosa* infections has remained stable in recent years; the prevalence of resistant strains has continued to rise [7]. Based on their antibiotic resistance profile, *P. aeruginosa* can be categorized into many phenotypes, as outlined below. The MDR phenotype refers to isolates that demonstrate resistance to multiple antimicrobial agents across at least three different antimicrobial classes. The extensively drug-resistant (XDR) phenotype refers to isolates that are resistant to many antimicrobial agents across all classes, except for two or fewer agents. Isolates that are pan-drug-resistant (PDR) are those that are not killed by any antimicrobial drug, regardless of the type of drug [8, 9]

In this study, we aimed to demonstrate the effects of antibiotic toxicity against resistant *P. aeruginosa* strains. Therefore, the standard growth curve has been used to detect this effect.

2 METHODOLOGY

2.1 BACTERIAL ISOLATES

A total of twenty-eight *Pseudomonas aeruginosa* isolates were collected from clinical specimens, eight from the Shaho laboratory and 20 from the burn and plastic surgery hospital in Sulaymaniyah. Four isolates were selected for further analysis based on their antibiotic resistance profiles. Ethical approval was obtained from faculty science and health at Koya University in 13/2/2025

2.2 IDENTIFICATION

Isolates were cultured on selective media, MacConkey agar, and cetrimide agar. Routine biochemical tests were performed on all samples to confirm *P. aeruginosa's* identity. The tests included Gram staining, oxidase, catalase, triple sugar iron (TSI) agar, Simmon's citrate, and motility tests. The VITEK 2 compact system with an ID-GNB card for the identification of bacteria was used to confirm the *P. aeruginosa* isolates [10, 11].

2.3 ANTIBIOTIC SUSCEPTIBILITY TEST

An antibiotic susceptibility test was performed on the VITEK 2 AST-N419 Card to determine the minimum inhibitory concentration (MIC) of different antibiotics. The card contained the following antibiotics: Amikacin, Gentamicin, Piperacillin/tazobactam, Imipenem, Meropenem, Ciprofloxacin, Ceftazidime and Cefepime. The AST-N419 Card was chosen for two reasons: first, it is recommended by the manufacturer for use against *P. aeruginosa*; second, it includes antibiotics from different antimicrobial classes [12].

2.4 DNA EXTRACTION

DNA extraction from the four selected isolates was performed using the AddPrep Genomic DNA Extraction Kit, according to the manufacturer's instructions. Briefly, 200 µL of the overnight bacterial culture was centrifuged at 13,000 rpm for 30 seconds to pellet the cells. The pellet was lysed with 200 µL of lysis solution, followed by the addition of Proteinase K solution (20 mg/ml). The mixture was vortexed and incubated at 56 °C until complete cell lysis was achieved. Subsequently, 200 µL binding solution was added, mixed by vortexing, and incubated at 56 °C for an additional 10 min. After adding 200 µL

absolute ethanol, the sample was transferred to a spin column for purification. Washing steps were performed using Washing Solutions 1 and 2 were performed, followed by elution with 100-200 µL Elution Solution [13].

2.5 DNA INTEGRITY AND PCR APPLICATION OF 16S RDNA

The quality of the extracted DNA and PCR product was checked using a Nanodrop spectrophotometer and gel electrophoresis by running 60 ng on 1% agarose gel for 60 min at 80 V. PCR was used to amplify 16S rDNA (~1515bp). The reaction consisted of 15 µL of 2X Add Taq Master (Addbio), 5 pmol (1 µL) of each forward (P1F-TGAAGAGTTTGATCATGGCTCAG) and reverse (P1R-TTCCCCTACGGTTACCTTGT) primer (rRNA gene), and 20ng (1 µL) extracted DNA. The volume was completed by adding 12 µL uclease-free water to a final volume of 30µl. Corbett thermal cycler was used for PCR reactions. The PCR amplicons were sent to South Korea (Macrogene Inc.) [14].

2.6 GROWTH CURVE ASSAY

The growth curves of the selected isolates were studied under both antibiotic-free and antibiotic-stress conditions. One of the four selected isolates was susceptible to all the tested antibiotics (A2) and was used as a control. Two isolates showed resistance to all antibiotics present (Amikacin, Gentamicin, Piperacillin/tazobactam, Imipenem, Meropenem, Ciprofloxacin, Ceftazidime and Cefepime) in VITEK 2 AST-N419 Card (A3 and A4), and the resistance of the fourth isolate was limited to three antibiotics (Piperacillin/tazobactam, Ceftazidime and Cefepime) (A9). The growth curve assay was conducted in flat-bottom 96-well microtiter plates, with a total volume of 200 µL per well. The design of the microtiter plate is shown in Table 1. Each column of the plate represented different experimental conditions.

The initial optical density for each well was adjusted to an absorbance of 0.05 at a wavelength of 630 nm. Antibiotic concentration varied depending on the resistance profile of each isolate. Antibiotic concentrations for isolates A3A and A4A were Amikacin 64 µg/mL, Gentamicin 16 µg/mL, Piperacillin/tazobactam 128/4 µg/mL, Imipenem 16 µg/mL, Meropenem 16 µg/mL, Ciprofloxacin 4 µg/mL, Ceftazidime 64 µg/mL and Cefepime 32 µg/mL. In case A9A, which was resistant to only three antibiotics, the concentrations used were piperacillin/tazobactam (128/4 µg/mL), ceftazidime (32 µg/mL), and cefepime (16 µg/mL). The antibiotics added to sterilized nutrient broth then the media dispensed into the 96-well microtiter plate using multichannel micropipette. The plates were incubated at 37 °C and agitated at 280 RPM. The optical density was measured at 630 nm every 15 min using an ELIZA reader [15, 16].

Table 1. Microtiter plate's inoculation layout design diagram.

	1	2	3	4	5	6	7	8	9	10	11	12
A	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
B	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
C	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
D	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
E	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
F	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
G	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		
H	M	MA	A2	A2A	A3	A3A	A4	A4A	A9	A9A		

Columns 1 and 2 Negative Controls: media only (M) and media + antibiotic only (MA). Columns 3 and 4: Isolate A2 with and without antibiotics (A2) and A2A. Columns 5 and 6: A3 with no antibiotics (A3) and with antibiotics (A3A). Columns 7 and 8: Without antibiotics (A4) and without antibiotics (A4A). Columns 9 and 10: Isolate A9 without (A9) and with (A9A) antibiotic.

2.7 STATISTICAL ANALYSIS

The data were assessed using IBM SPSS Statistics v25 and GraphPad Prism 8. One-way ANOVA along with Tukey's multiple comparison tests was used to compare the means OD630 values under different conditions (normal and antibiotic stress conditions).

3 RESULTS

3.1 IDENTIFICATION

Bacterial growth on MacConkey and cetrimide agar was not unique to *P. aeruginosa*, as other species such as Enterobacter, Morganella, and Proteus were also present. Therefore, standard biochemical tests (Table 2 and figure 1) were used, followed by confirmation using VITEK 2 identification. The colony morphology of *Pseudomonas aeruginosa* was examined on both

Cetrimide and MacConkey agar. On Cetrimide agar, colonies appeared greenish due to pyocyanin production, while on MacConkey agar, they formed non-lactose fermenting, pale colonies.

Table 2. The characteristics and biochemical tests of *P. aeruginosa* isolates

Biochemicals	Features
Pigmentation	Diffusible Green pigment on Nutrient and muller-Hinton agar Blue-green pigment on cetrimide after 24 h
Gram Stain	Negative
Motility	Motile
Morphology	Single, small Bacilli
Oxidase	Positive
Citrate utilization	Positive
Catalase	Positive
H_2S production	Negative

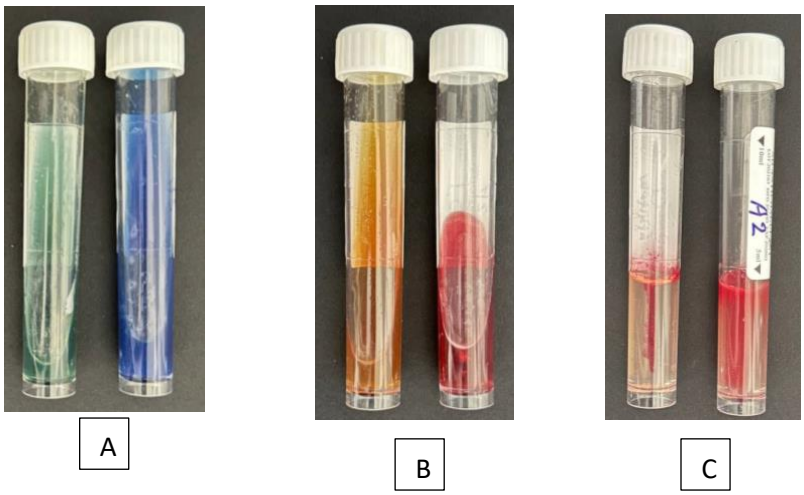

FIGURE 1. The biochemical tests: (A) Simmons's citrate- Green indicates -ve while blue indicates +ve (*P. aeruginosa* is +ve), (B) Triple Sugar Iron (TSI) agar – the right tube shows *P. aeruginosa* (slant alkaline (red), but alkaline (red), no gas and H2S) and (C) motility test -the right shows no motility, while left shows motility indicated by diffuse of bacterial growth from the stab line.

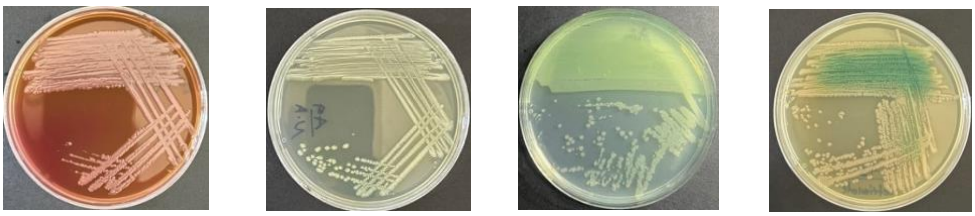

FIGURE 2. Bacterial growth on (A) MacConkey agar - that are pale or colourless colonies due to the inability of *P. aeruginosa* to ferment lactose. (B) Muller-Hinton Agar - large irregular colonies that appear greenish. (C, D) cetrimide agar – greenish-blue colonies due to pyocyanin production.

3.2 ANTIBIOTIC SUSCEPTIBILITY TEST

For detecting the resistance pattern and MIC of isolates, VITEK 2 AST-N419 has been used, and depending on that, four strains of *P. aeruginosa* were selected (A2, A3, A4, and A9). The MIC are summarized in Table 3. Isolate A2 was susceptible to all antibiotics, isolates A3 and A4 were resistant to all antibiotics, and isolate A9 was resistant only to Piperacillin/tazobactam, Ceftazidime and Cefepime.

Table 3. MIC (µg/mL) of Strains by VITEK 2

Antibiotics	MIC (µg/mL)	Interpretation
Amikacin	A2: 4	Susceptible (S)
	A3: ≥64	Resistant (R)
	A4: ≥64	Resistant (R)
	A9: 4	Susceptible (S)
Gentamicin	A2: ≤1	Susceptible (S)
	A3: ≥16	Resistant (R)
	A4: ≥16	Resistant (R)
	A9: ≤1	Susceptible (S)
Piperacillin/tazobactam	A2: 8	Susceptible (S)
	A3: ≥128	Resistant (R)
	A4: ≥128	Resistant (R)
	A9: ≥128	Resistant (R)
Imipenem	A2: ≤0.5	Susceptible (S)
	A3: ≥16	Resistant (R)
	A4: ≥16	Resistant (R)
	A9: 2	Susceptible (S)
Meropenem	A2: ≤0.25	Susceptible (S)
	A3: ≥16	Resistant (R)
	A4: ≥16	Resistant (R)
	A9: 1	Susceptible (S)
Ciprofloxacin	A2: ≤0.06	Susceptible (S)
	A3: ≥4	Resistant (R)
	A4: ≥4	Resistant (R)
	A9: 0.25	Susceptible (S)
Ceftazidime	A2: 2	Susceptible (S)
	A3: ≥64	Resistant (R)
	A4: ≥64	Resistant (R)
	A9: 32	Resistant (R)
Cefepime	A2: 2	Susceptible (S)
	A3: ≥32	Resistant (R)
	A4: ≥32	Resistant (R)
	A9: 16	Resistant (R)
Ceftazidime/avibactam	A2: 2	Susceptible (S)
	A3: ≥16	Resistant (R)
	A4: ≥16	Resistant (R)
	A9: 2	Susceptible (S)
Ceftazidime/tazobactam	A2: 1	Susceptible (S)
	A3: ≥32	Resistant (R)
	A4: ≥32	Resistant (R)
	A9: 2	Susceptible (S)

MIC: Minimum inhibition concentration. Values represent the average of three independent replicates. µg/mL: microgram per milliliter

3.3 DNA INTEGRITY AND PCR APPLICATION OF 16S RDNA

The integrity of the extracted DNA and PCR products was assessed by running 60 ng DNA on a 1% agarose gel for 60 min at 80 V. The PCR products demonstrated good quality, as the result indicated by distinct bands at approximately ~1515 bp (Figure 3).

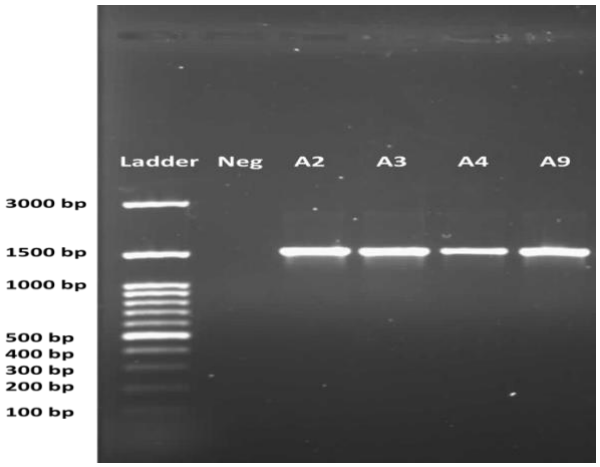

FIGURE 3. Gel electrophoresis of PCR products for 16S rDNA verification. Lanes Ladder and Neg represent a 100bp (100bp-3000bp) DNA marker (Genedirex) and a negative control that has been run without a DNA template. All of the PCR product bands align at ~1500 bp, indicating successful amplification of the 16s rRNA gene (~1500 bp) 16S rDNA. A2 is P. aeruginosa isolate (susceptible to all tested antibiotics), A3 and A4 P. aeruginosa isolates (resistant to all tested antibiotics), and A9 P. aeruginosa isolate (resistant to Piperacillin/tazobactam, Ceftazidime and Cefepime).

3.4 16S RRNA GENE

BLAST was used to analyze the 16S rDNA genes of our strains (A2, A3, A4, and A9) against the NCBI nucleotide database to determine their taxonomy. The results indicated that all strains shared a high sequence identity with *P. aeruginosa* (Table 4). A2 and A9 were 100% identical to *Pseudomonas aeruginosa* strains Pw3 (MN006645.1) and B2 (MG009432.1), respectively. However, A3 exhibited 98.87% identity with *Pseudomonas aeruginosa* strain EPPAS 1 (PP216568.1), whereas A4 displayed 99.45% identity with *Pseudomonas aeruginosa* strain B81 (CP142449.1). These outcomes prove that all four isolates belonged to *Pseudomonas aeruginosa* with minor genetic variation at the strain level.

Table 4. Identity present along with accession numbers of identical strains.

Strain ID	Description	Present identity	Accession Number
A2	*Pseudomonas aeruginosa* strain Pw3 16S ribosomal RNA gene, partial sequence	100.00%	MN006645.1
	Pseudomonas aeruginosa strain OFAA14 16S ribosomal RNA gene, partial sequence	100.00%	MT353653.1
	Pseudomonas aeruginosa strain PRK1 16S ribosomal RNA gene, partial sequence	100.00%	MT704553.1
	Pseudomonas aeruginosa strain NIOT.SNWCZ5 16S ribosomal RNA gene, partial sequence	100.00%	PP515656.1
A3	*Pseudomonas aeruginosa* strain EPPAS 1 16S ribosomal RNA gene, partial sequence	98.87%	PP216568.1
	Pseudomonas aeruginosa strain B81 chromosome	98.87%	CP142449.1
	Pseudomonas aeruginosa strain 60503 chromosome, complete genome	98.87%	CP041774.1
	Pseudomonas aeruginosa strain T-1 16S ribosomal RNA gene, partial sequence	98.87%	JN000304.1
A4	*Pseudomonas aeruginosa* strain B81 chromosome	99.45%	CP142449.1
	Pseudomonas aeruginosa strain 60503 chromosome, complete genome	99.45%	CP041774.1
	Pseudomonas aeruginosa strain CZI1002861 chromosome, complete genome	99.45%	CP145538.1
	Pseudomonas aeruginosa strain PA15_L5_37.22_ST773_VIM2 chromosome, complete genome	99.45%	CP143906.1
A9	*Pseudomonas aeruginosa* strain B2 16S ribosomal RNA gene, partial sequence	100.00%	MG009432.1
	Pseudomonas aeruginosa strain 6404_1 16S ribosomal RNA gene, partial sequence	100.00%	EF556270.1
	Pseudomonas aeruginosa strain FQP29 16S ribosomal RNA gene, partial sequence	100.00%	MF144517.1
	Pseudomonas aeruginosa strain AVP17 16S ribosomal RNA gene, partial sequence	100.00%	KF527831.1

3.5 GROWTH CURVE ASSAY

Figure 5 presents the growth curve of each isolate grown under normal (antibiotic-free) and antibiotic-stress conditions. The selection of these isolates facilitated a comparison between susceptible, multidrug-resistant, and extensively drug-resistant *P. aeruginosa* isolates. A2 was susceptible to all antibiotics; therefore, as a control for other strains along with A2, we used media (M) and media and antibiotics (MA) without bacteria and negative control. The growth curve showed steady growth of A2 (M = 0.57258 ± 0.028574, SE) over time, while M and MA did not show any change in absorbance. One way ANOVA was used to analyze the OD630 values, and the results revealed a significant difference among the tested conditions ($F(6) = 46.353$, p-value 0.0001; Table 5) The A3 strain was subjected to normal (M = 0.62187 ± 0.024463, SE and antibiotic stress conditions (M = 0.50985 ±0.025433, SE), and the results suggested a slower and less dense growth pattern that did not inhibit growth under antibiotic stress conditions. The A4 (M = 0.64953 ± 0.026740, SE) strain displayed similar trends as A3 but

experienced greater inhibition under antibiotic stress (M = 0.50985 ±0.025433, SE). A9 exhibited a distinct response. Although resistant to individual antibiotics, its growth was notably inhibited when exposed to a combination of these antibiotics, with absorbance not exceeding 0.2.

Table 5. One-way ANOVA and Tukey's Post Hoc test for different bacterial isolates and under different conditions (normal and antibiotic stress conditions).

Bacteria & Conditions	Mean ± SD	SE	Tukey's Post Hoc (Significant Pairwise Comparisons)
A2	0.5726 ± 0.3051	0.0286	A2 vs. A4A (P = 0.019), A2 vs. A3A (P= 0.506), A2 vs. A9A (P < 0.001)
A3	0.6219 ± 0.2612	0.0245	A3 vs. A3A (P = 0.016)
A3A	0.5099 ± 0.2716	0.0254	-
A4	0.6495 ± 0.2855	0.0267	A4 vs. A4A (P < 0.001)
A4A	0.4622 ± 0.2670	0.0250	-
A9	0.5967 ± 0.2514	0.0235	A9 vs. A9A (P < 0.001)
A9A	0.1752 ± 0.0390	0.0037	-
Between-group variance: F(6, 791) = 46.353, P < 0.0001			

Tukey's post hoc analysis revealed that OD630 values in the control (A2) were significantly higher than A4A (P= 0.019) and A9A (P < 0.001) both of which were subjected to antibiotic stress (see Figure 4). However, no significant value was observed between A2 and other bacteria and conditions (A3, A4, A3A and A9). Additionally, The A3, A4 and A9 resulted in significantly higher growth rate (OD630 values) compared to A3A, A4A and A9A (P= 0.016, P < 0.001 and P < 0.001, respectively

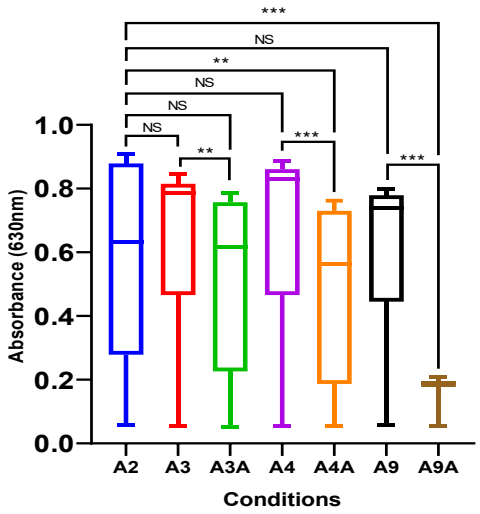

FIGURE 4. Graphic illustration for the isolates by one way ANOVA using GraphPad. A2, which was sensitive to all antibiotics, was tested against all conditions and a significant P-value was observed when compared to A4A (P = 0.019) and A9A (P<0.001). Each isolate was analyzed between normal and antibiotic stress conditions, with all comparisons showing significant differences A3 vs A3A P = 0.016, A4 vs A4A P <0.001 and A9 vs A9APp <0.001

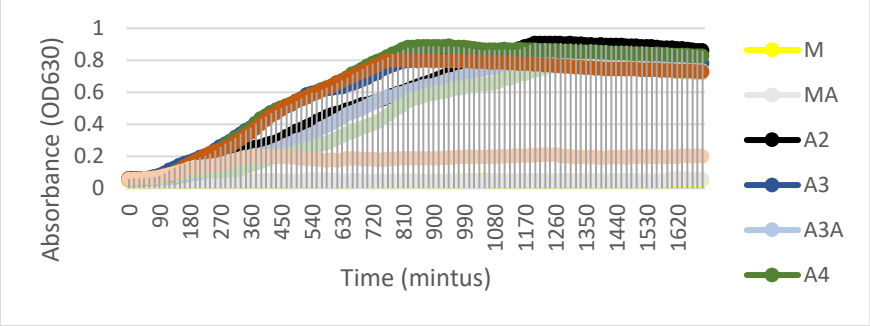

FIGURE 5. Growth curve of *Pseudomonas aeruginosa* isolates A2, A3, A4, and A9 under antibiotic stress. Optical density (OD630) measurements were recorded every 15 minutes for 27 hours. The untreated cultures (A2 (black), A3 (dark blue), A4 (dark green), A9 (dark orange)) exhibited characteristic sigmoid growth (S-shape) and reached stationary after 900 minutes. On the other hand, antibiotic-treated strains (A3A (light blue), A4A (light green), A9A (light orange)) did grow slower and A9A had the most inhibition. The controls (M (yellow), MA(gery)) consistently low OD values throughout. The one-way ANOVA revealed the differences between the groups were significant (F (6, 791) = 46.353, p< 0.0001). Post hoc analysis showed significant pairwise differences between groups. Notably, A2 vs. A4A (P = 0.019), A2 vs. A9A (P < 0.001), A3 vs. A3A (P = 0.016), A4 vs. A4A (P < 0.001), A9 vs. A9A (P < 0.001). The findings show that antibiotic stress has a significant inhibitory effect on bacterial growth particularly A9A.

4 DISCUSSION

This study demonstrated the impact of antibiotic susceptibility on growth characteristics of multidrug-resistant *Pseudomonas aeruginosa* isolates from clinical samples. Resistant strains of *P. aeruginosa* pose an increasing challenge, and are a major clinical issue associated with high morbidity and mortality [17].

The identity of the isolates was tested using standard biochemical tests and confirmed using the VITEK 2 compact system. The molecular diagnosis of 16S rDNA gene was used for further confirmation (Figure 3). We used both automated and molecular diagnosis for confirmation, as some of the isolates were not *P. aeruginosa*. Several studies suggest that standard biochemical tests alone are not sufficient for the diagnosis of P aeruginosa [18, 19]

Four isolates were selected based on their susceptibility to antibiotics: A2 was found to be sensitive to all antibiotics and served as a control, A3 and A4 were resistant to all antibiotics, and A9 was resistant to only three antibiotics. Using the VITEK 2 compact system (Table 3) for antibiotic susceptibility testing provided fast and reliable results, which confirmed the resistance patterns of the isolates. Many studies have corroborated the validity of this methodology in detecting resistant organisms and providing therapeutic options [20].

The test identified variations in the isolates' responses to antibiotics, including their growth patterns (Figure 5). A2 (M = 0.57258 ± 0.028574, SE) grew faster than the other strains on an antibiotic-free medium. In comparison, isolates A3 and A4 showed lower growth rates under antibiotic stress (M = 0.50985 ±0.025433, SE and M = 0.50985 ±0.025433, SE respectively), proving that these strains may be resistant but affected to some extent by antibiotics during the test. Additionally, A4 was more affected than A3 was [21]. These data complement other studies showing that even growth rates of resistant strains can slow due to exposure to antibiotics. This is likely a function of the metabolic burdens or stress responses induced by antibiotics [22, 23].

Interestingly, isolate A9, which was resistant to Piperacillin/tazobactam, Ceftazidime and Cefepime, showed a reduction in growth when exposed to these antibiotics. This suggests that β-lactam antibiotics (such as piperacillin/tazobactam, ceftazidime, and cefepime) may work synergistically when used together. Previous studies have shown that this combination can improve antibiotic uptake Nd lead to bacterial (*P. aeruginosa*) elimination [24]. To confirm this synergy, further research using checkerboard assays or time-kill kinetics would be valuable, potentially guiding combination therapies for multidrug-resistant infections [25].

Although antibiotics had a significant effect on bacterial growth, particularly in A3 vs A3A (P = 0.016) and A4 vs A4A (P < 0.001), complete growth inhibition was not observed. These findings highlight important clinical implications. As *P. aeruginosa* is becoming resistant to multiple drugs, there is a need to rethink the treatment of infections. Susceptibility testing should be part of the regular management of infections caused by this organism. This information indicates that certain combination therapies can effectively overcome resistance.

5. Conclusion

This study highlights how multidrug-resistant *P. aeruginosa* can survive despite antibiotic treatment, including the complexity of its resistance mechanisms. Although antibiotics have a significant effect, bacterial growth was not completely inhibited. The antibiotic synergy observed in isolate A9 suggests that combination therapy might be an effective way to combat resistance, making it a promising area for further research. Future studies should aim to identify specific resistance genes through whole-genome sequencing and investigate synergistic effects using in vitro methods such as checkerboard and time-kill assays. Gaining a deeper molecular understanding of these resistance mechanisms will be essential for developing more effective treatments for MDR *P. aeruginosa* infections. Future studies should explore alternative therapeutic strategies, including the application of bacteriophages and the development of novel antimicrobial agents. In addition, genetic studies such as transcriptome analysis on resistant isolates are required for future studies that will explain the possible genetic mechanism with the development of various therapies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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