Journal of Biomedical Sciences | ISSN: 2960-9356
Research Article Peer-review, Open Access

Molecular Characterization of Multidrug-Resistant *Pseudomonas Aeruginosa* from Retail Meat and its Lytic Bacteriophage

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Abstract

MRSA-resistant Pseudomonas aeruginosa in commercial meat is a big concern for veterinary public health. In Al-Diwanyia, Iraq, 150 beef samples were collected for this study. P. aeruginosa was found to be 33.33% of the germs discovered out of 60% ($P < 0.001$). All isolates tested negative for penicillin, ceftazidime, and cefepime against a disk diffusion panel of eleven antibiotics. Identification was further confirmed by molecular and phylogenetic analysis. To address this threat, a lytic bacteriophage was successfully isolated to target these MDR strains. The phage rapid adsorption rate 0.267 ± 0.002 at 5 min and a short eclipse period of 15 minutes. the lysis time of the phage against Pseudomonas aeruginosa was monitored over a 3-hour period a reduction in bacterial growth (Abs at 600 nm) was observed after 1.5 h, which corresponded with an increase in phage activity (Abs at 350 nm). The highest phage activity was recorded at 3 h (0.245 ± 0.003). These results support the phage-based interventions as a sustainable and effective biocontrol strategy to reduce microbial risks in meat products and enhance food safety.

Keywords: Bacteriophage; Biocontrol; Multidrug-resistance; Pseudomonas aeruginosa; Retail meat.

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Introduction

Meat is a very important source of high quality animal protein and other important nutrients to human bodies, but it offers a good growth condition to microbes since the meat contains high amount of moisture and nutrients. Meat can be contaminated with various spoilage and pathogenic microorganisms during slaughter, processing, handling, and in storage, profoundly influencing the safety and shelf life factors of meat and the quality of its organoleptic properties (Williams, 2007; Rouger *et al*., 2017). Most notable of this group of microorganisms has become the strains of *Pseudomonas aeruginosa*, which are

prevalent spoilage organisms during aerobic storage but, more so, during chilled storage (Wickramasinghe *et al*., 2019). *Pseudomonas aeruginosa*, a rod-shaped, non-fermentative, and gram-negative bacterium has a versatile metabolism and an ability to persist in the environment. It also results in proteolytic and lipolytic enzymes and volatile nitrogenous substances like ketones, esters, and aldehydes that lead to unpleasant conversion of meat flavor, texture, and color (Papadopoulou *et al*., 2020). It has been known to thrive in different environmental conditions such as low temperatures, thus ensuring dominance of meat spoilage flora and shortening the shelf life of meat (Ercolini *et al*., 2009). Besides its culinary versatility, *P. aeruginosa* is also an interesting opportunistic pathogen and can cause infections in both humans and animals and is well known by its innate resistance to various antibiotics, primarily due to its lower rate of outer membrane permeability and strong active transport pumps (Bassetti *et al*., 2017). With the rising interest regarding antimicrobial resistance, other methods of controlling bacterial contamination in food, especially in the meat and meat products have been of interest. The utilization of bacteriophages capable of inducing bacterial cell lysis is the most promising strategy. Bacteriophages are natural, very specific, and self-replicating biocontrol agents that do not influence a host physiology or a useful microbiota (Benie *et al*., 2017; Franzetti & Scarpellini, 2007). In contrast to chemical antimicrobials or wholesale antibiotics, phages have the potential to go after certain bacterial species like *P. aeruginosa* without restructuring much of the microbiome in food systems. Studies conducted in recent years have shown that bacteriophages are effective in the control of *Pseudomonas spp*. in various food matrices, such as meat, fish and poultry (Benie *et al*., 2017; Doulgeraki *et al*., 2012). The use of phages in meat processing has demonstrated the potential to decrease the bacterial load, retard spoilage, and support food safety without having any impact on the physicochemical characteristics of the particular food product (Fletcher *et al*., 2018). In addition, phage therapy is in line with the current trends in the preservation of natural foods and the consumer desire to buy chemical-free foods, as it is sustainable, and it leaves no residues. Research on the prevalence and molecular detection of *P. aeruginosa* in meat in Iraq has summarized its high occurrence in beef, chicken and mutton, particularly in the retail meat markets in some cities within Iraq, such as Mosul. They found that the generality of *P. aeruginosa* was confirmed in meat samples using PCR-based identification of the *16S rRNA* and *rpoB* genes (Jawher & Hassan, 2022). This was a matter of public health concern and the future trend remains a challenge in terms of proper measures to curb this menace. But there is little local evidence on the lytic possibility of bacteriophages against that type of isolates. Thus, the current research is focused on, testing the lytic activity of bacteriophages against *P. aeruginosa* strains isolated from retail meat samples in Al-Diwaniyah city, Iraq.

2. Materials and Methods

2.1. Sample Collection

A total of 150 retail meat samples, including beef (n=50), chicken (n=50), and minced meat (n=50), were gathered for this study. The collection method was aseptic, utilizing sterile knives and gloves to avoid contamination. The 25-g samples were deposited straight into labeled sterile Whirl-Pak stomacher bags.

Our Al-Diwaniyah lab transported all bags in refrigerated cartons at 0–4°C to preserve microbiological integrity. Following ISO 17604:2015 criteria, the distribution mechanism was closely controlled to keep within two hours. We processed the samples immediately or held them at 4°C until we began the microbiological examination in the lab.

2.2. Isolation and Identification of *P. aeruginosa*

To prepare for isolation, each specimen's 25 g beef was combined with 225 mL of BPW. We next inoculated the combinations onto Blood Agar and MacConkey Agar plates and incubated them at 37°C for 24 hours. Any suspicious colonies were subcultured on Cetrimide Agar (Hi Media, India) to isolate P. aeruginosa (Rezaloo et al., 2022). Each plate was then incubated at 37°C for two days. Counting the colonies after incubation, we selected those for identification. Catalase, Oxidase, and Triple Sugar Iron tests were employed to identify bacteria in accordance with microbiological methods (Cappuccino &

Sherman, 2020).

A drop of 3% hydrogen peroxide (H_2O_2) was introduced to a new bacterial colony sample before the catalase test on a clean glass slide. The reaction was observed immediately to assess the presence of catalase activity (Al-Zubaidi,2021; Hassan, 2020).

Oxidase test: A sterile applicator stick was used to transfer a colony onto filter paper impregnated with oxidase reagent. The test area was observed for color changes within the time recommended by the manufacturer to determine oxidase activity (Al-Tamimi, 2022).

TSI Test: Triple Sugar Iron agar test was performed to assess the ability of the isolates to ferment glucose, lactose, and sucrose, and to detect gas or hydrogen sulfide production. TSI slants were prepared according to the manufacturer's instructions, sterilized, and allowed to solidify in a slanted position. Each tube was inoculated by first stabbing the butt of the medium on with a sterile needle and then streaking the surface of the slant. The inoculated tubes were incubated at 37°C for 18–24 hours. After incubation, the slants were examined for color changes in both the slant and the butt, as well as the presence of any gas bubbles or black precipitate indicating H_2S production (Forbes *et al*., 2017).

2.3. Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of *P. aeruginosa* isolates was determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA) (CLSI,2023; EUCAST, 2024), in accordance with (CLSI, 2023) and (EUCAST, 2024) criteria. The bacterial suspensions were calibrated using a 0.5 McFarland turbidity standard (about 1.5×10^8 CFU/mL) before being evenly injected onto the MHA surface. We applied antibiotic disks aseptically and incubated plates at 37°C for 18–24 hours. Millimeter-wide inhibitory zones designated isolates as susceptible (S), intermediate (I), or resistant (R) after incubation. Interpretive tables were used.

2.4. Molecular Characterization and Phylogeny

Genomic DNA was isolated using a Jena Bioscience kit according to the instructions. Polymerase chain reaction amplified 16S rRNA using EconoTaq® PLUS GREEN Master Mix. The thermal cycling regimen began with 5 minutes of denaturation at 94°C, followed by 35 cycles of denaturation, annealing, and extension at 65–68°C for 30 seconds and 72°C for 1 minute. Another 10-minute 72°C period was added. Electrophoresis on a 1.5% agarose gel under UV illumination confirmed PCR results. The amplicons were validated and sequenced using Sanger sequencing. After aligning in BioEdit with ClustalW, BLASTn compared the sequences to NCBI. To analyze MEGA 11 evolution, a Neighbor-Joining phylogenetic tree was created with 1000 bootstrap iterations.

2.5. Bacteriophage Isolation and preparation

The bacteriophages were aseptically isolated from municipal wastewater treatment plants. Samples were centrifuged at 4,000-5,000 × g for 10 minutes to remove debris and germs. Phage filtrate was filtered via 0.22 μm membrane filters (Millipore, USA) to exclude bacteria. The filtrate was cultured at 37°C for 18-24 hours with an overnight P. aeruginosa culture to boost phage titers and enrichment before analysis. (Zaher & Salem, 2014).

2.6. Biological Characterization of phage

Disk diffusion, microliter plate tests, and double agar overlay assessed lytic activity. Plaque morphology was considered. Clear inhibitory zones of 10–28 mm width appeared on plates after 24–48 hours at 37°C. (Jorgensen & Turnidge, 2007).

- Adsorption was assessed using phage (10^6 PFU/mL) and host bacteria (10^8 CFU/mL) for 5 minutes, whereas penetration was evaluated similarly. According to Hyman and Abedon (2009), unadsorbed phages were measured by spinning the solution at 5,000 × g for 10 minutes. The DNA injection penetration time was determined after 37°C adsorption. (Abedon, 2011).

- To assess replication during eclipse, chloroform was given at 30-second intervals to liberate intracellular virions. After 15 minutes, the eclipse ended. (Abs: 0.251) (Kropinski et al., 2009).
- The latent period of lysis was measured by monitoring infected cultures at 37°C for OD_ {600}} and OD_ {350}}. After one hour, the bacterial population decreased, indicating progeny phage release. (Kutter & Sulakvelidze, 2005**).**
- Thermal Stability: Phage stability was assessed by exposing aliquots to temperatures ranging from 4°C to 70°C for one hour. Optimal stability and activity were observed at 40°C (Abs: 0.125).

2.7. Statistical Analysis

One-way ANOVA statistical test was used to assess the significance of the differences among different experimental groups. In the case of a significant F-value, the LSD multiple comparisons test was carried out as a post-test to compare the means in different groups. The SPSS program was used for this purpose. Results with $P < 0.05$ were considered as statistically significant (Daniel & Cross, 2018).

3. Results

3.1. Prevalence and MDR Profile of Pseudomonas aeruginosa

A total of 150 retail meat samples were processed for microbiological screening. From these samples, 90 bacterial isolates were successfully recovered, representing a recovery rate of 60%. Among the identified isolates, Notably *Pseudomonas aeruginosa* was the most prevalent pathogen, accounting for 33.33% (n = 30) of the total recovered isolates. The distribution of the recovered bacterial species is (**Table 1)**. The variations in prevalence among the different bacterial groups were statistically highly significant (P <0.001).

Table 1. Distribution of bacterial isolated by species.

Bacterial Species	**Number**	**Percentage**
E. coli	24	26.66
Proteus spp.	8	8.88
Staphylococcus aureus	12	13.33
Staphylococcus epidermidis	16	17.77
Pseudomonas aeruginosa	30	33.33
Total	90	100
P value	**<0.001(HS)**	

Antibiotic susceptibility testing was performed on a panel of 11 antibiotics. Inhibition zone diameters were measured and used to categorize isolates as susceptible, intermediate, or resistant according to standard interpretive criteria (P<0.0001). The distribution zone diameters for each antibiotic are summarized in (Table 2).

Table 2. Zone diameter distribution and resistance patterns of *P. aeruginosa* isolates to different antibiotics

Antibiotic	**>30 Highly Susceptible**	**26-30 Susceptible**	**21-25 Intermediate**	**16-20 Resistant**	**11-15 Highly Resistant**	**≤10 Completely Resistant**
MEM	2(6.66)	26(86.66)	0(0)	0(0)	0(0)	2(6.66)
AK	6(20)	16(53.33)	4(13.33)	2(6.66)	2(6.66)	0(0)
PC	0(0)	0(0)	0(0)	0(0)	0(0)	30(100)
TPZ	0(0)	0(0)	8(26.66)	8(26.66)	4(13.33)	10(33.33)
ATM	0(0)	0(0)	0(0)	2(6.66)	16(53.33)	12(40)
IPM	0(0)	0(0)	16(53.33)	6(20)	4(13.33)	4(13.33)

GM	0(0)	0(0)	0(0)	14(46.66)	4(13.33)	12(40)
TOB	0(0)	0(0)	14(46.66)	6(20)	0(0)	10(33.33)
CIP	16(53.33)	2(6.66)	2(6.66)	4(13.33)	0(0)	6(20)
CAZ	0(0)	0(0)	0(0)	0(0)	0(0)	30(100)
FEP	0(0)	0(0)	0(0)	0(0)	0(0)	30(100)
Calculate X^2	**624.24**					
Calculate P value	**<0.0001(HS)**					

3.2. Molecular Identification and Phylogenetic Analysis

Using the BLAST to analyze sequences against the NCBI GenBank database, we confirmed presence of *P. aeruginosa species* in the analyzed meat samples. **(Figure 1)** shows the PCR amplification products for the identified isolates. Several isolates showed high sequence identity reaching up to 99.81% with Pseudomonas aeruginosa. Additional species identified included *P. frederiksbergensis*, *P. marginalis*, and *P. otitidis*. Some sequences also exhibited similarity to uncultured Pseudomonas strains as well as related genera, particularly *Azotobacter* and *Ectopseudomonas*. The lengths of sequences were between 49 and 528 base pair (**Figure 2).** This of multidrug-resistant *P. aeruginosa* isolates and indicate the presence of diverse Pseudomonas species in the analyzed meat samples**.**

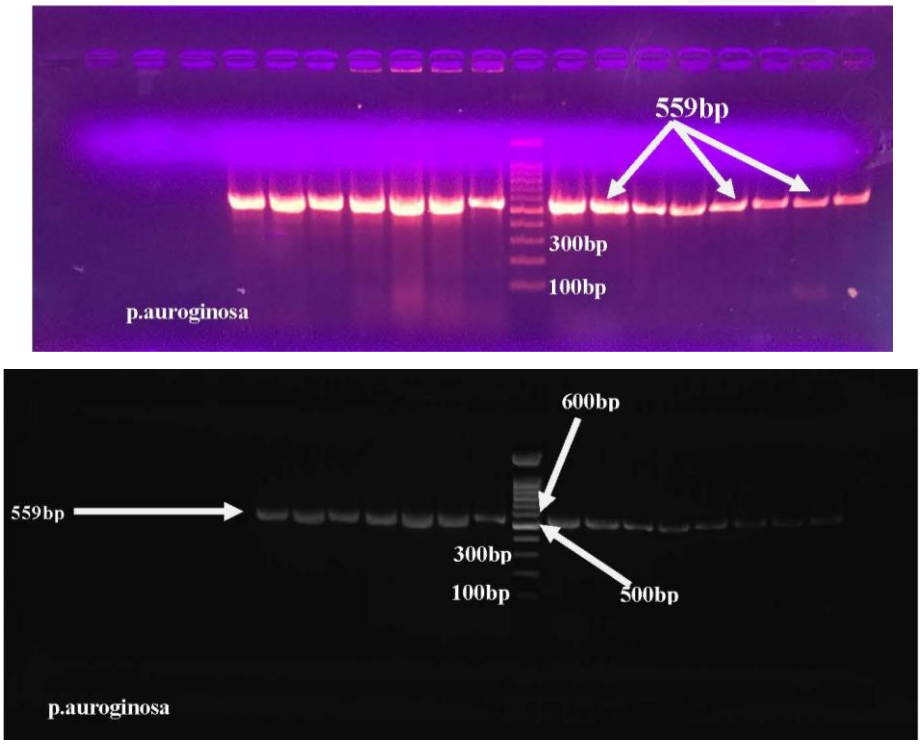


Figure 1. Agarose gel electrophoresis of PCR amplification products of *P. aeruginosa.*

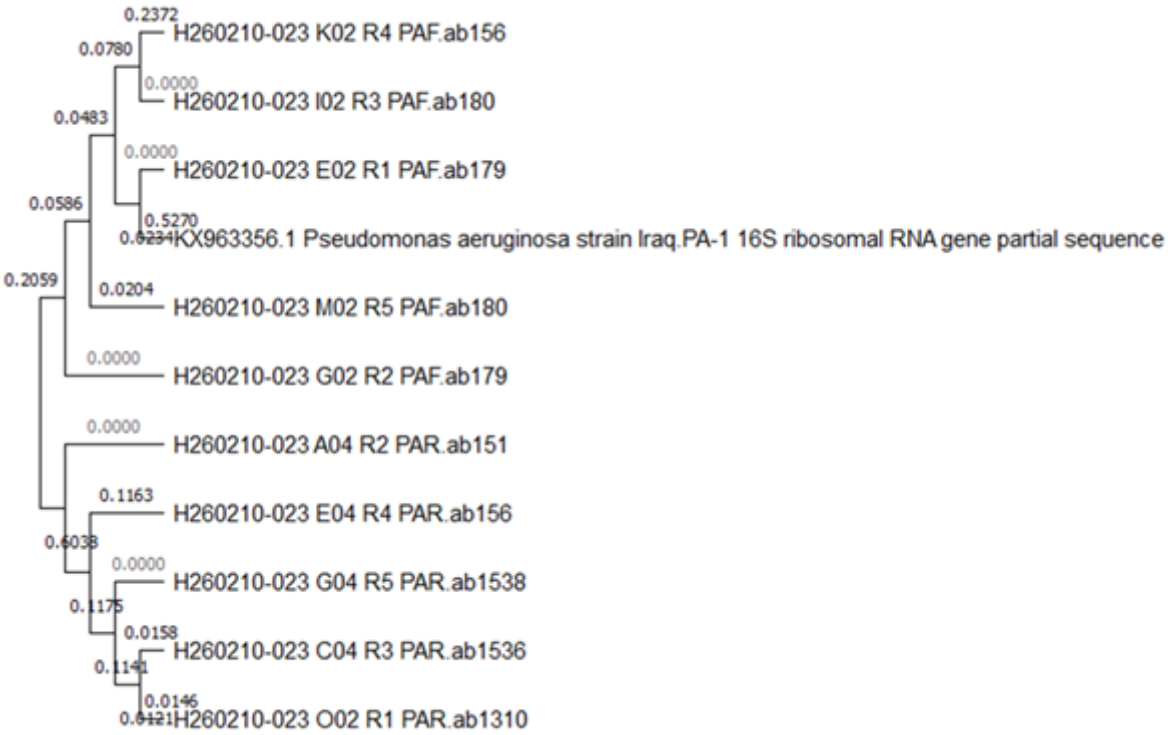


Figure 2. Phylogenetic tree showing the relationship of the MDR *P. aeruginosa* isolates with reference strains *pseudomonas species* based on *16S rRNA* sequences.

3.3. Isolation and Morphological Characterization of Bacteriophage

Bacteriophage isolation from sewage water samples led to the successful isolation of lytic bacteriophages against *P. aeruginosa*. This phage produced clear, circular plaques on bacterial lawns, shown in **Figure 3.** The presence of these clear plaques indicates a high lytic efficiency of the isolated bacteriophages against the target host.

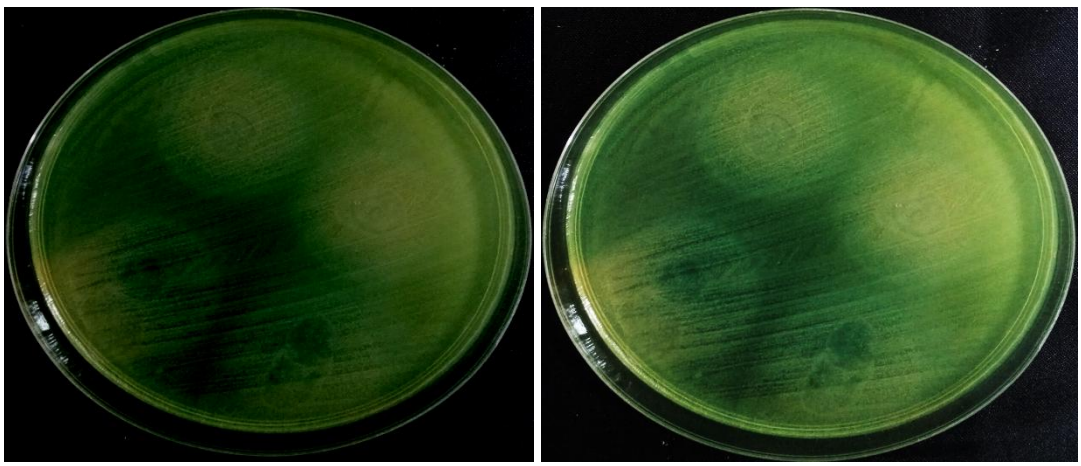


Figure 3. the lytic bacteriophages morphology on a lawn of *P. aeruginosa* (plaques).

3.4. Adsorption Kinetics

The phage showed a high adsorbed to host cells, reaching a maximum adsorption rate of 0.267 ± 0.002 at 5 min (**Table 3, Figure 4)**. The bacterial culture reached a peak OD600 of 0.831 ± 0.001, The level of growth indicates robust host growth.

Table 3. Adsorption rate of bacteriophage particles on *P. aeruginosa* cells.

Time(min)	Adsorption rate (mean ±SE)
0 min	0.186±0.001
1 min	0.235±0.001
3 min	0.245±0.003
5 min	0.267±0.002
10 min	0.216±0.003
20 min	0.247±0.001
30 min	0.214±0.0023
LSD(P<0.05)	**0.007**

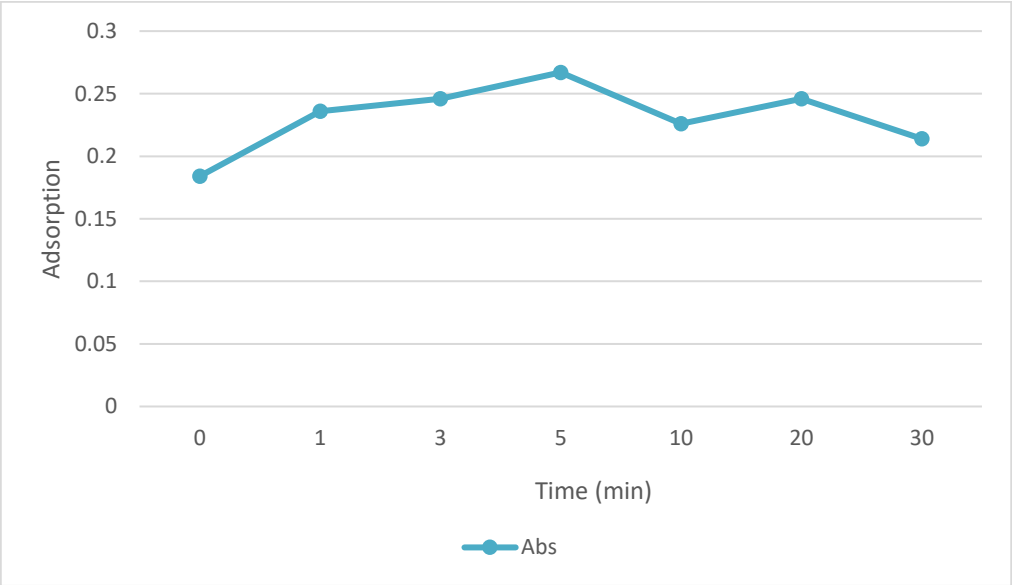


Figure 4. Adsorption rate the isolated bacteriophage to *Pseudomonas aeruginosa* cells.

3.5. Eclipse Phase

The phage showed a rapid eclipse phase, reaching a maximum absorbance of 0.251 at 15 min (**Figure 5).** The curve showed a sharp initial incline within the first 5 min, followed by a stable plateau phase, efficient viral replication prior to host cell lysis.

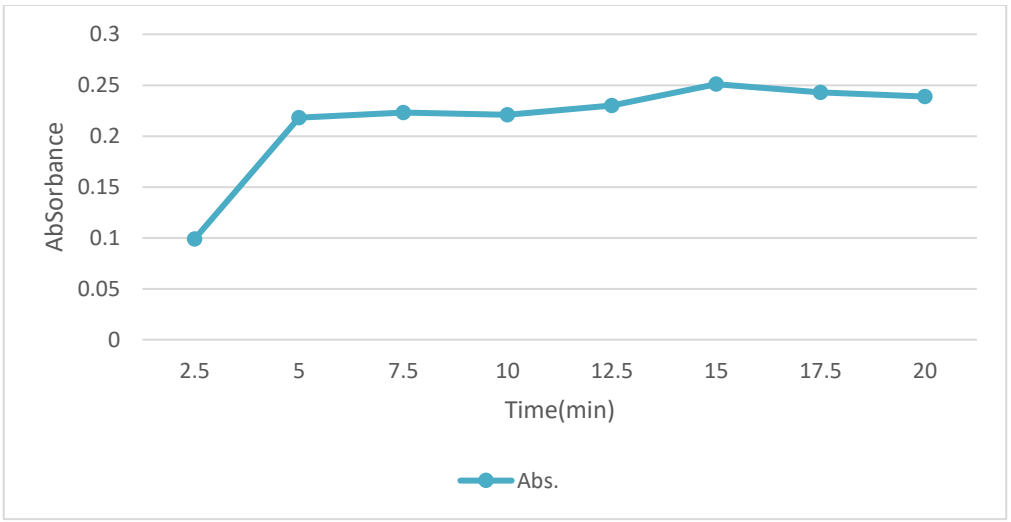


Figure 5. the eclipse phase for the isolated bacteriophage.

3.6. Thermal Stability and Lysis time of the Isolated Phage

The thermal stability of the isolated bacteriophage was evaluated at temperatures ranging from 4 °C to 70 °C **(Figure 6).** The phage exhibited the highest lytic activity at 40 °C (0.125). Lytic activity was maintained between 4 °C and 60 °C, whereas a noticeable decrease was observed at 70 °C (0.024).

The lytic activity of the phage against *Pseudomonas aeruginosa* was monitored over a 3-hour period (**Table 4).** A reduction in bacterial growth (Abs at 600 nm) was observed after 1.5 h, which corresponded with an increase in phage activity (Abs at 350 nm). The highest phage activity was recorded at 3 h (0.245±0.003).

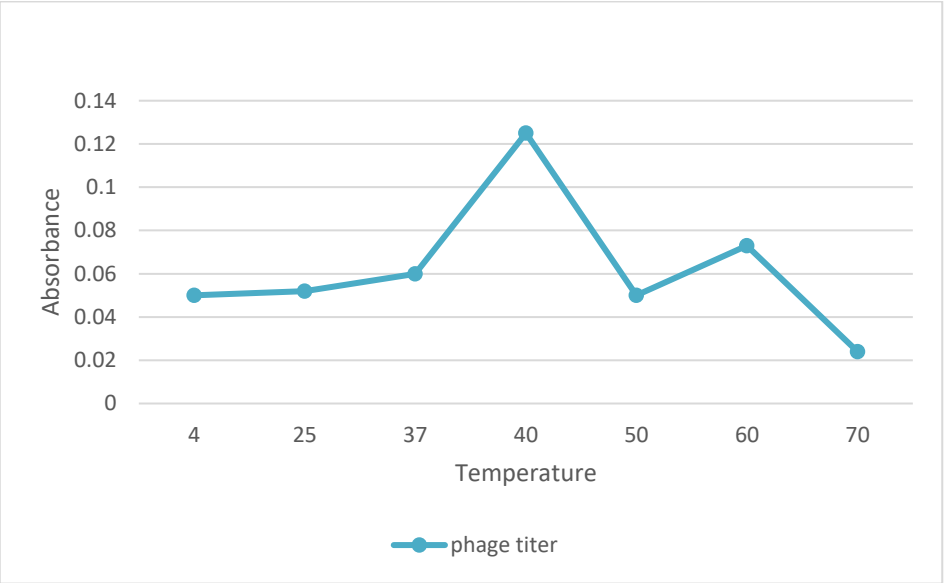


Figure 6. Thermal stability profile of the isolated bacteriophage across a temperature range of 4 °C to 70 °C

Table 4. Lysis time of the bacteriophage against *Pseudomonas aeruginosa* over a 3-hour period

Time	Abs. 350 nm	Abs. 600 nm.
30 min.	0.602±0.004	0.286±0.001
1hrs.	0.275±0.002	0.560±0.001
1.5 hrs.	0.102±0.001	0.318±0.003
2 hrs.	0.636±0.001	0.364±0.001
2.5 hrs.	0.729±0.006	0.447±0.001
3hrs.	0.831±0.001	0.507±0.002
LSD(P<0.05)	**0.010**	**0.005**

3.7. Susceptibility of bacterial isolates to phage infection

The host range of the isolated bacteriophage was evaluated against several *P. aeruginosa* isolates using the spot test assay. Different lysis zones on the tested isolates' bacterial lawns demonstrated high lytic activity (Figure 7). The lysis zone size and clarity of several P. aeruginosa strains demonstrated that the isolated bacteriophage may be effective against a broad spectrum of clinical and environmental infections. With an average diameter of 12.6 mm, lysis zone quantitative evaluation was effective. Table 5 shows that No. 1, with a zone diameter of 28 mm, was the most sensitive. The others had sizes of 10 to 14 mm.

Table 5.

number	diameter(mm)
1	28
2	11
3	10
4	12
5	12
6	12
7	14
8	13
9	12
10	12
12	10
17	13
18	11
19	10
56	12

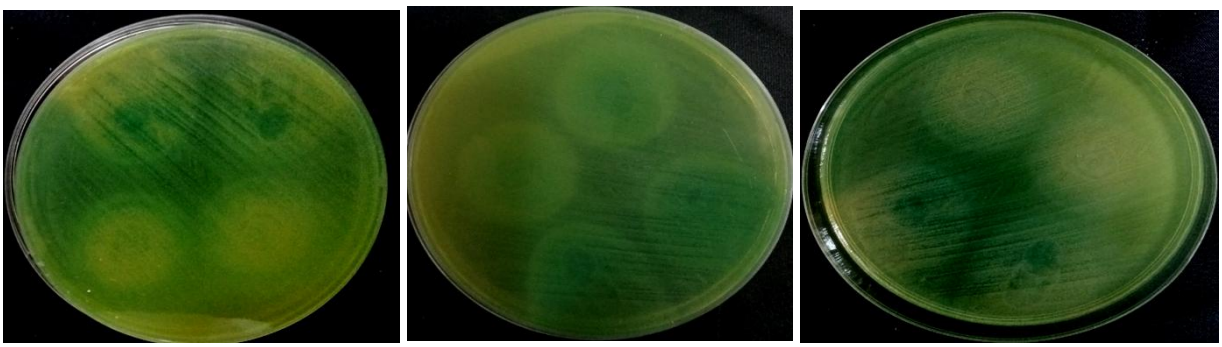


Figure 7. Susceptibility of bacterial isolates to phage infection, isolated bacteriophage against *Pseudomonas aeruginosa* isolates (showing clear lysis zones in the spot test assay).

Discussion

A 60% bacterial recovery rate was found in retail meat samples in Al-Diwaniyah, with 33.33% finding P. aeruginosa. The higher occurrence of food hygiene issues in local retail environments is noteworthy (\bm {P < 0.001}). Jawher and Hasan (2022) observed significant Pseudomonas aeruginosa contamination in Mosul, Iraq, supporting our findings. According to Li et al. (2023), P. aeruginosa is a widespread but unappreciated biofilm-forming bacteria in food preparation and deliberately abundant in meat. Biofilms allow bacteria to thrive on numerous surfaces, according to Badawy et al. (2023). Veterinary MDR strains transmit to humans by this continuous channel. Biofilms persist in slaughterhouses, thus traditional sanitation procedures may not be enough to eliminate them.

High recovery rates from different meats indicate supply chain issues. This supports Rezaloo et al.'s (2022) claim that meat is a primary reservoir for this opportunistic virus. According to the One Health philosophy, this is the first harmful item after pollution that causes illness. Finding P. aeruginosa in retail meat is concerning since it can cause significant opportunistic infections in vulnerable people. Al-Fraji et al. (2020) say poor bacterial load management in retail causes food poisoning outbreaks, notably in Iraq. This necessitates a more integrated disease monitoring system from production to consumption.

The trial's drug resistance profile is concerning. All penicillin, ceftazidime, and cefepime inoculations failed. Some bacteria are entirely resistant to third- and fourth-generation cephalosporins, posing a public health risk. These drugs are prescribed for severe Pseudomonas infections in hospitals. Our data confirm

the global MDR pandemic reported by Guibert et al. (2025) and Yilmaz et al. (2026). Retail meat has highly resistant bacteria, suggesting that the veterinary industry utilizes antibiotics frequently and possibly without control. Ahmed (2022) says these isolates' resistance genes show they've learned to avoid typical treatments. Abdullah et al. (2024) noted that increased chicken meat medication resistance threatens the region's population's health.

Our molecular characterisation demonstrates that MDR bacteria are both passive contaminants and disease-causing. Poursina et al. (2023) and Rasti et al. (2022) examined biofilm growth and virulence features including antibiotic resistance. Multidrug-resistant and biofilm-producing bacteria like P. aeruginosa are difficult to remove with traditional antimicrobials. This reinforces the necessity for a One Health policy, since Abdel-Rhman and Rizk (2021) noted the need for better PCR-based typing to monitor these hazardous isolates. They may infect other bacteria in water or soil due to their environmental resistance, worsening the resistance situation.

Isolating a lytic bacteriophage was a major step in creating a "integrative" long-term risk reduction technique. Short eclipse length of 15 minutes and high adsorption rate of 267 at 5 minutes were phage biological characteristics. Time is crucial in food systems, therefore kinetic qualities are key for biocontrol. Like Abdelghafar et al. (2023), we discovered that lytic phages were efficient against resistant Pseudomonas lineages. Harada et al. (2022) and Reyneke et al. (2020) found that our phage suppresses many bacteria, including drug-resistant ones, with an inhibition zone bigger than 28 mm. It could be a "phage cocktail" that lyses various strains, boosting protection.

Industrial phages must be thermally stable at 60°C. Rather of utilizing chemicals that could compromise quality, a robust phage can handle the multiple temperature variations during meat preparation. Gummadi (2024) and Sathe et al. (2025) say this is essential for food-grade phage products. The growth curve shows bacterial proliferation declines after 1.5 hours, showing potential. Phages eliminate the pathogen of interest while preserving the meat's flavor and beneficial microbes, according to Elbarbary and Abdelmotilib (2023) and Brás et al. (2024).

The phage's ability to kill drug-resistant pathogens suggests it could fight "superbugs." Chegini et al. (2020) found that phage therapy could make meat safer. This safeguards buyers, improves animal welfare, and reduces antibiotic resistance's environmental impact. This comprehensive plan addresses Iraqi and global microbiological issues via One Health. Switching to biological controls like phages is crucial as antibiotics lose efficacy.

Conclusion

This study highlights a global public health concern associated with retail beef: the enduring presence of multidrug-resistant (MDR) Pseudomonas aeruginosa. The global ineffectiveness of standard treatments is underscored by the emergence of antibiotic resistance to essential medications such as Ceftazidime and Cefepime. This is a "One Health" issue because antibiotic-resistant bacteria can quickly get into the food web. Farmers all around the world can use isolating lytic bacteriophages as a realistic and long-term solution. Phage Q10 is a great biocontrol agent since it has a quick lytic cycle, can withstand heat, and can kill MRSA without changing the taste of food. Integrative biological therapies are becoming more and more necessary to use to protect people's health around the world and make sure food is safe.

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