ISOLATION AND IDENTIFICATION OF MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* FROM MUTTON MEAT IN BAGHDAD: FOCUS OF AZURIN GENE

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is considered to be the primary cause of food spoilage and foodborne illnesses, with significant implications for public health due to its multidrug resistance (MDR). This study examined the prevalence and resistance profiles of *Pseudomonas aeruginosa* in 75 imported mutton samples from Baghdad. The bacteria were detected in 17.3% of the samples and exhibited resistance to multiple antibiotic classes, including beta-lactams, cephems, monobactams, and fluoroquinolones. The presence of MDR *Pseudomonas aeruginosa* in imported meat poses a significant risk to public health, potentially facilitating the spread of antimicrobial-resistant infections through the food supply chain. This study underscores the urgent need for strict sanitary practices, regular monitoring of microbial contamination, and robust antimicrobial resistance surveillance to ensure the safety of food products and protect public health.

Keywords: *Pseudomonas aeruginosa*, imported meat, multidrug resistance (MDR), Azurin gene, food safety.

1. Introduction:

Globally, meat is the main source of protein. Mutton is a nutrient-dense source of vitamins, proteins, fatty acids, and minerals. Mutton can be transformed into many animal products, including salami, kebabs, burgers, and sausage. Researchers suggest that meat constitutes a significant component of the global diet[1]. Approximately $900 billion is lost annually due to food spoilage globally. Given these challenges, scientists are increasingly focused on ensuring the safety of meat products, as their perishable nature makes them particularly susceptible to contamination and spoilage. Meat provides animal proteins for human nourishment and serves as a favourable environment for an increase of different microorganisms. Contamination can occur during dressing, and carcasses that are first dressed may be exposed to illness-causing contamination that may persist during meat preservation and processing[2].

In addition to contamination during slaughter, meat processing equipment can serve as another critical source of contamination. Equipment surfaces, if not properly sanitized, can harbor bacteria that

transfer to meat during processing, compounding the risk of spoilage and foodborne illnesses. Improperly cleaned equipment has been shown to act as a reservoir for spoilage microorganisms and pathogens[3]. Meat and meat processing equipment are highly susceptible to typical foodborne bacteria and microorganisms that cause meat deterioration due to their perishable nature. Meat and meat products deteriorate due to specific bacteria like *Pseudomonas* spp., lactic acid bacteria (LAB) such as *Lactobacillus* spp. and *Carnobacterium* spp., and *Brochothrix thermosphacta*[4.] *Pseudomonas* strains are particular spoilage microorganisms that are seen in beef, chicken, and fish[5;6].

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogenic bacterium present in various environments, including water, soil, air, as well as plant and animal tissues. *Pseudomonas aeruginosa*, produced by pyocyanin dyes, a blue-green pigment, is a key virulence factor with diverse roles in its pathogenicity[7]. *Pseudomonas aeruginosa* frequently causes spoilage in foods with high water content and diets high in nutrients[8]. *Pseudomonas* is identified as a distinct bacteria responsible for contaminating meat[9] and dairy products[10]. *Pseudomonas aeruginosa* bacteria, which are resistant and virulent, are considered the primary causes of food spoilage and foodborne diseases[11]. *Pseudomonas aeruginosa* is an opportunistic microorganism responsible for the enteric disease[12].

Pseudomonas aeruginosa is a cold-tolerant bacterium capable of surviving in food stored under refrigeration temperatures (0–7 °C). Therefore, minimizing the initial levels of *Pseudomonas* spp. in meat is crucial to limit their proliferation and reduce spoilage. The growth of *Pseudomonas* significantly affects the organoleptic properties of meat, leading to product loss before consumption[^13]. Certain strains of spoilage bacteria, including *Pseudomonas*, are commonly found in poultry, seafood, and meat. At the point of spoilage, these bacteria produce off-flavors due to nitrogenous compounds that generate volatile chemicals such as ketones, aldehydes, and esters[14;5]. Furthermore, the strong antibiotic resistance of *Pseudomonas*, attributed to the transparency of its outer membrane lipoprotein, poses a serious health risk to consumers.

According to recent studies, *Pseudomonas aeruginosa* isolates are highly resistant to various antimicrobial agents[16.] In 2017, *Pseudomonas aeruginosa*, which was resistant to treatment, caused over 32,000 infections and resulted in 2,700 fatalities among hospitalized patients in the USA. *P. aeruginosa* is the most resistant to aminoglycosides, tetracyclines, penicillin, quinolones, cephalosporin, macrolides, and β-lactam antibiotics[17.] Antibiotic resistance has become a critical global issue with significant implications for public health. Strains like *P. aeruginosa* are increasingly resistant to first-line treatments, creating challenges for clinicians in managing infections. This resistance can lead to

prolonged hospital stays, higher medical costs, and increased mortality rates[18].

The strong antibiotic resistance of *Pseudomonas* is a serious health concern, partially attributed to its outer membrane lipoproteins and the expression of specific genes, such as the azurin gene. This gene encodes a blue copper protein involved in electron transfer, which not only contributes to the bacterium's virulence but may also play a role in its resistance mechanisms[19]. The Azurin gene is a type of cupredox protein that is found in the periplasmic and cytoplasmic spaces. It is also known as blue-copper protein and has been shown to have many biological activities[20]. The Azurin protein enables the bacterium to evade the immune system by inducing p53-mediated apoptosis in macrophages, thereby helping the bacteria avoid immune responses. [21] described how the structure of the Azurin protein resembles the variable domains observed in immunoglobulin group members.

This study focuses on assessing the incidence of *Pseudomonas aeruginosa* in imported mutton samples from Baghdad and evaluating its antimicrobial resistance profiles. The findings aim to provide valuable insights into the role of imported meat in spreading MDR bacteria, emphasizing the need for enhanced monitoring and stricter food safety practices to mitigate risks to public health.

MATERIALS AND METHODS:

Samples

The study was conducted in the Baghdad area. Meat samples were collected from several marketplaces and butcher shops. Seventy-five imported mutton meat samples were randomly collected under sterile conditions from Shaab, Qahira, Hayy-Ur, New Baghdad, and Sadr City. Collected samples were transported immediately to the College of Veterinary Medicine, Veterinary Public Health Laboratory. The samples were collected using sterile tools and placed in sealed containers to minimize external contamination during transportation. The sample was kept at **4°C** in insulated coolers with ice packs to inhibit bacterial growth. Additionally, secondary containment was used to prevent leaks or exposure. Aseptic techniques were applied upon arrival at the lab, including scraping the external layer to remove potential surface contaminants.

Isolation and identifications

A stomacher ground 25 mg of the sample, and the sample was cultured in TSA broth (Tryptone soy broth) for 18 hours at 37°C. After that, cultures were grown on MacConkey agar and Tryptone soy agar for 18–24 hours (Domanska and Rozanska., 2003), and the growth of bacteria was seen. The diagnosis was confirmed using specific media and biochemical tests. The isolation and biochemical confirmation procedures were conducted in the Department of Veterinary Public Health and Meat Hygiene Laboratory at the College of Veterinary Medicine. The isolates were confirmed by molecular identification of *P. aeruginosa* utilizing the Azurin gene.

Antibiotic susceptibility assessment

To evaluate the susceptibility of *Pseudomonas aeruginosa* isolates to antibiotics. The Kirby-Bauer method, also known as simple disk diffusion, was

employed to examine the antibiotic resistance pattern in *Pseudomonas aeruginosa* isolates. *Pseudomonas aeruginosa* isolates were grown for 18 hours in Tryptone Soya broth (TSB) medium at 37°C aerobically for 1 day, Following the suspension of bacteria preparation at a 0.5 McFarland concentration (ranging from 1×10^8 to 2×10^8 CFU/ml), the isolates were cultured on Mueller-Hinton agar while various antibiotic discs were distributed via media included, Amikacin (AK) 30ug, Ampicillin (Amp) 10ug, Aztreonam (AT) 30 ug, Ticarcillin+ Clavulanic (TIC) 75/10 ug, Cefepime (CEP) 30 ug, Ceftazidime (CAZ) 30 ug, Ciprofloxacin (CIP) 5 ug, Colistin sulfate (CO) 25 ug, Gentamycin (GEN) 10ug, Imipenem (IMI) 10ug, Levofloxacin (LEV) 5 ug, Ofloxacin (OFX) 10 ug. Then, each medium was incubated at 37 °C for 24 hours. By determining and recording the susceptibility or resistance to the corresponding antibiotic according to the dimension of the zone of growth inhibition surrounding every disk, the antibiotic resistance pattern of the isolates was evaluated. The experiment utilized the *Pseudomonas aeruginosa* standardized strain (ATCC 10145) as a control positive. The findings were analyzed according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing[24]. The results were categorized as sensitive, moderate, or resistant based on the comparison of inhibitory zone widths following the manufacturer's guidelines.

Phylogenetic analysis, DNA sequencing, and tree construction

Genomic DNA was extracted from isolated samples using the G-spin Genomic Extraction Kit (Intron, Korea). Additionally, DNA was extracted by centrifuging 1–2 ml of bacterial cells at 13,000 rpm for 1 minute, discarding the residue [28]. Following this, 300 µl of G-Buffer solution was added, mixed, and incubated at 65°C for 15 minutes with periodic rotation. Then, 250 µl of B-Buffer was added and thoroughly mixed, and the lysate was loaded onto a column centrifuge at 13,000 rpm [29]. The column was washed sequentially with 500 µl of Wash Buffer-A and Wash Buffer-B, centrifuging after each wash. DNA was eluted by adding 50–200 µl of elution buffer, incubated at room temperature for 1 minute, and centrifuged at 13,000 rpm [25].

The extracted DNA was verified on a 1% agarose gel stained with Red Safe Nucleic Acid Stain (Intron, Korea) and visualized under UV light at 320 nm [26]. Primers for the Azurin gene were used: F (ATGCTACGTAAACTCGCTGC) and R (GCTTTTTCATGCAGCGGAT), with a molecular weight of 467 bp. PCR solutions were prepared using GoTaq® G2 Green Master Mix, and the PCR protocol included initial denaturation at 94°C for 5 minutes (1 cycle), followed by 35 cycles of denaturation at 94°C for 30 seconds and annealing at 56°C for 30 seconds, and a final extension at 72°C for 5 minutes. PCR products were analyzed using gel electrophoresis (Cleaver, UK) [27].

Sequence of Azurin gene and Phylogenetic tree

The target azurin gene was sequenced using the PCR product of *Pseudomonas aeruginosa*, which was transmitted to Macrogen Corporation in Korea. The phylogenetic tree was then built using the maximum composite likelihood and minimum evolution method by Molecular Evolutionary Genetics Analysis (MEGA) version 6.0. This software removed all positions with gaps and missing data after the sequence data were aligned using BLAST (Basic Local Alignment Search Tool) at the National Centre for Biotechnology

Information database (NCBI) and searched for a similar sequence previously published in the NCBI[22].

Statistical analysis

All statistical analyses were performed using the Statistical Analysis System[23] software. Quantitative data were expressed as means ± standard deviation (SD). To compare mean values between different groups, the t-test was applied, enabling the identification of statistically significant differences in antibiotic susceptibility patterns [30].

The Chi-square test (χ^2) was used to assess associations between categorical variables, specifically to compare the isolation rates of *Pseudomonas aeruginosa* from different regions in Baghdad. The chi-square test was also applied to determine the distribution of bacterial isolates from imported mutton meat samples [31]. Statistical significance was considered at $P \leq 0.05$ and $P \leq 0.01$, depending on the specific analysis. The degrees of freedom were calculated based on the number of categories being compared. Furthermore, Pearson's correlation coefficient (r) was calculated to assess the strength and direction of linear relationships between continuous variables [32].

All statistical tests were conducted at a 95% confidence level, and results with p-values ≤ 0.05 were considered statistically significant unless otherwise specified [33].

RESULT

***Pseudomonas aeruginosa isolated*:**

Molecular identification of *Pseudomonas aeruginosa* was performed by confirming the presence of the Azurin gene using conventional PCR. A total of 75 imported meat samples were collected from Baghdad, specifically from Al-Qahira, Hayy-Ur, Sider City, Al-Shaab, and New Baghdad (15 samples per region) [34]. Thirteen isolates of *Pseudomonas aeruginosa* were identified, with the highest isolation percentage of *P. aeruginosa* was 4 (26.6%) isolated from both Hayy-Ur and Al-Qahira (**Table 1, Figure 1**). Statistical analysis revealed no significant differences in the distribution of *P. aeruginosa* among the different regions (χ^2 = 0.961, *P* =0.961).

Table (1): Isolation rates of *Pseudomonas aeruginosa* from different regions in Baghdad.

Area	Number of samples	Isolation % (15)
AL-Qahira	15	4 (26.6%)
Hayy-Ur	15	4 (26.6%)
Sider city	15	3 (20%)
AL-Shaab	15	1 (6.66%)
New Baghdad	15	1 (6.66%)
Total	75	100%
Chi-square test –χ^2 (P-value)	---	0.961 NS (279)
NS: Non-Significant.		

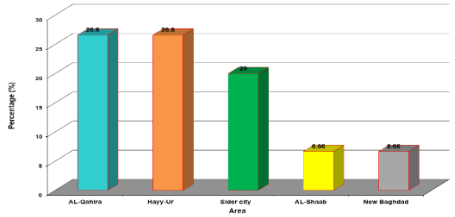

Figure (1) Isolation rates of *Pseudomonas aeruginosa* from different regions in Baghdad.

Colonies of *Pseudomonas aeruginosa* displayed distinct morphological characteristics on different growth media. On cetrimide agar, the colonies exhibited a glossy, smooth, and convex morphology with coloration ranging from greenish to yellow. In contrast, on MacConkey agar, *Pseudomonas aeruginosa* colonies appeared as large, white, milky, mucoid, and rounded, resembling pearls (**Figure 2**).

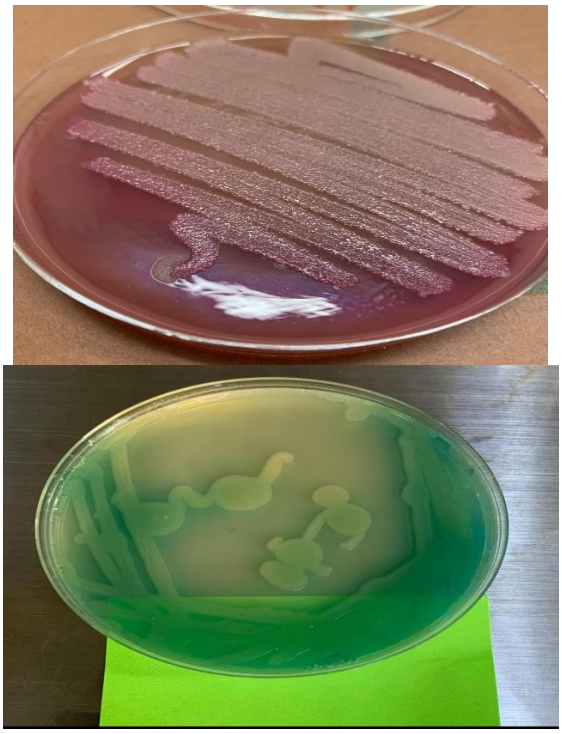

Figure (2) Morphological characteristics of *Pseudomonas aeruginosa* on (A) MacConkey agar appear as white-milky large mucoid and rounded pearls. (B) Cetrimide agar appears to have a large, irregular mucoid texture and yellow-green to blue pigmentation.

Bacterial isolation:

A total of 75 imported mutton meat samples were analyzed for bacterial contamination, revealing six distinct bacterial species. The distribution of isolates was as follows: *Escherichia coli* (43 isolates, 57.3%), *Pseudomonas aeruginosa* (13 isolates, 17.3%), *Salmonella* spp. (6 isolates, 8%), *Proteus* spp. (2 isolates, 2.7%), *Shigella* spp. (1 isolate, 1.3%), and *Aeromonas* spp. (1 isolate, 1.3%). The highest prevalence was observed for *E. coli*, accounting for more than half of the isolates (57.3%), followed by *Pseudomonas aeruginosa* (17.3%). Rare isolates included *Shigella* spp [35]. and *Aeromonas* spp., each representing only 1.3% of the total samples. Statistical analysis using the Chi-square test confirmed significant differences in bacterial prevalence (χ^2 = 22.90, $P \leq 0.01$), indicating that contamination patterns were non-random and significantly associated with sample conditions [36] (**Table 2, Figure 3**).

Table (2): Distribution of bacterial isolates from imported mutton meat samples in Baghdad.

Bacterial isolation	Number	Percentage
P. aeruginosa	13	17.3
E. coli	43	57.3
***Shigella* spp.**	1	1.3
***Salmonella* spp.**	6	8
***Proteus* spp.**	2	2.7
***Aeromonas* spp.**	1	1.3
Total	75	100%
Chi-square test –χ^2 (P-value)	---	22.905 ** (0.0001)
**** (P≤0.01).**		

**: Highly Significant (P≤0.01)

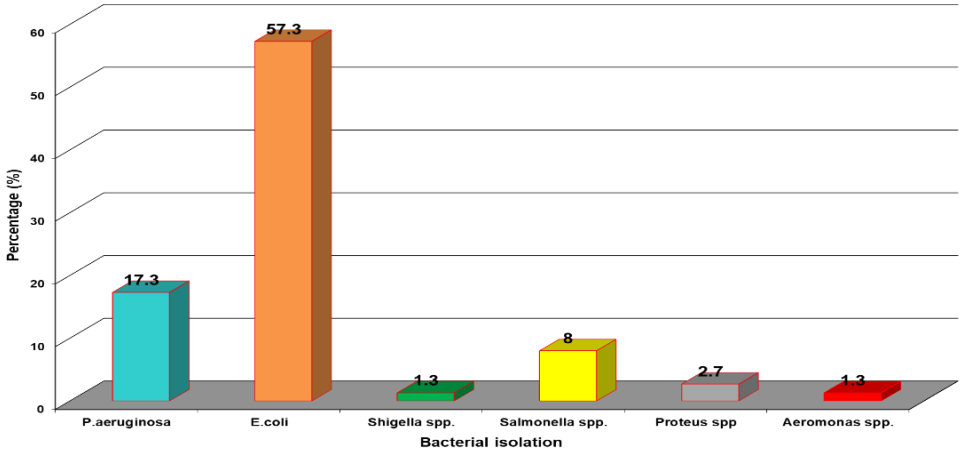

Figure (3) Distribution of bacterial isolates from imported mutton meat samples in Baghdad.

Antibiotic resistance

Out of the 13 *Pseudomonas aeruginosa* isolates from mutton meats, the antimicrobial resistance profile demonstrated significant resistance of isolates to Ampicillin, Ceftazidime, and Ofloxacin (100%), Ticarcillin-Clavulanic acid, (92.3%), Aztreonam (76.92%), Levofloxacin (69.23%), Ciprofloxacin (61.53%), and Cefepime (53.84%) [37], In contrast, the *Pseudomonas aeruginosa* sensitive to Colistin sulphate (92.3%), Gentamycin (76.92%), Amikacin (53.84%) and Imipenem (46.15%). Specific isolates exhibited multi-drug resistance to Amoxicillin, Aztreonam, and Meropenem at different degrees. (**Table 3).** The findings underscore the concern of widespread antibiotic resistance, particularly against commonly used broad-spectrum antibiotics. These results highlight the need for continuous monitoring and the implementation of effective antimicrobial stewardship programs.

Table (3): Antibiotic susceptibility profile of *Pseudomonas aeruginosa* isolates from imported mutton meat in Baghdad

Antibacterial agent		Code	Mg/disk	S		I		R		P—value
				Nu	%	Nu	%	Nu	%	
1	Amikacin	AK	30	7/13	53.84	1/13	7.69	5/13	38.46	0.0001 **
2	Ampicillin	Amp	10	0/13	0	0/13	0	13/13	100	0.0001 **
3	Aztreonam	AT	30	1/13	7.69	2/13	15.38	10/13	76.92	0.0001 **
4	Ticarcillin+ Clavulanic	TIC	75/10	1/13	7.69	0/13	0	12/13	92.3	0.0001 **

5	**Cefepime**	CEP	30	4/13	30.76	2/13	15.38	7/13	53.84	0.0001 **
6	**Ceftazidime**	CAZ	30	0/13	0	0/13	0	13/13	100	0.0001 **
7	**Ciprofloxacin**	CIP	5	5/13	38.46	0/13	0	8/13	61.53	0.0001 **
8	**Colistin sulphate**	CO	25	12/13	92.3	0/13	0	1/13	7.69	0.0001 **
9	**Gentamycin**	GEN	10	10/13	76.92	1/13	7.69	2/13	15.38	0.0001 **
10	**Imipenem**	IMI	10	6/13	46.15	2/13	15.38	5/13	38.46	0.0001 **
11	**Levofloxacin**	LEV	5	3/13	30.76	1/13	7.69	9/13	69.23	0.0001 **
12	**Ofloxacin**	OFX	10	0/13	0	0/13	0	13/13	100	0.0001 **
P-value			--	--	0.0001 **	--	0.0001 **	--	0.0001 **	---
** (P≤0.01).										

****: Highly Significant (P≤0.01)**

PCR technique

Detected of the Azurin gene

Twelve out of thirteen *Pseudomonas aeruginosa* isolates showed positive results for the Azurin gene, representing an 84.61% detection rate among the imported mutton meat samples. Which suggests a high prevalence of *Pseudomonas aeruginosa* carrying the Azurin gene in the tested samples (**Figure 4).**

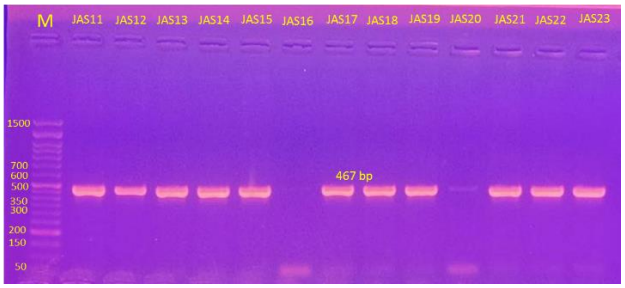

Figure (4) PCR amplification of the partial region of the Azurin gene in *Pseudomonas aeruginosa*.

DNA Sequencing, Phylogenetic Analysis and Tree Construction

Macrogen Corporation in Korea received the *Pseudomonas aeruginosa* PCR product to sequence the target azurin gene. Results of azirine gene sequencing and phylogenetic data analysis showed that the Iraqi isolate of *Pseudomonas aeruginosa* from a mutton meat sample is 100% identical to the corresponding region in the *Pseudomonas aeruginosa* strain PAAK088 deposited in the NCBI database (**Figure** 5).

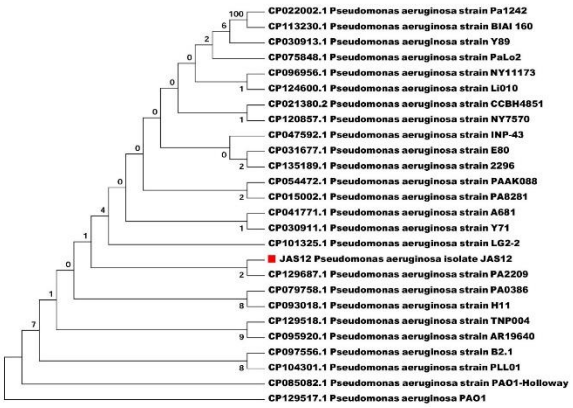

Figure (5). A phylogenetic tree showing the genetic similarity of the recovered *P. aeruginosa* strain with other strains in the GenBank database is labeled Red.

DISCUSSION

Pseudomonas aeruginosa isolated from Baghdad province

The results profile showed the isolation of 13(17.3%) *Pseudomonas aeruginosa* from a total of 75 mutton meat samples, Isolated of Baghdad from regions AL-Qahira, Hayy-Ur, Sider city, AL-Shaab, and New (15 from each region). Baghdad. Isolation percentages of positive *Pseudomonas aeruginosa* from selected regions in Baghdad province, in which the highest isolation percent of *P. aeruginosa* from Hayy-Ur and AL-Qahira 4 (26.6%) from 15 mutton meat samples (**Table 1).** These findings suggest a potential issue with hygiene practices during the handling, preparation, and storage of meat in Baghdad. The presence of *Pseudomonas aeruginosa* may indicate contamination at various stages of processing, transmission, and storage in supermarkets, which unstable electrical sources in Iraq can exacerbate. These conditions create an environment conducive to the proliferation of pathogenic microorganisms, leading to the risk of infections upon consumption (Al-Obaidi and Dawood, 2023).

However, the small sample size may limit the generalizability of the findings, as it might not fully represent the contamination levels across all meat markets in Baghdad. Additionally, there is a possibility of cross-contamination during sample collection and processing, which could affect the accuracy of the isolation results. Factors such as improper sterilization of tools, handling errors, or environmental contamination in laboratories may contribute to false-positive results. Implementing stricter aseptic techniques and expanding the sample size in future studies would help minimize these risks and enhance the reliability of the findings.

Bacterial isolation

The present finding revealed that 75 imported mutton meat samples expressed 13(17.3%) *Pseudomonas aeruginosa*, 43(57.3%) *E. coli*, 1(1.3%) *Shigella spp*., 6(8%) *Salmonella spp.,* 2(2.7%) *Proteus spp*., and 1(1.3%) *Aeromonas spp*. (**Table 2, Figure 3).** All these particular bacterial isolates were pathogenic to humans; these results may support the opinion that animal-derived food is a significant cause of foodborne diseases. (WHO., 2010).

These results agree with findings reported by Feath (2019), who detected various pathogenic bacteria in both frozen and raw retail bovine meat. Their study revealed the presence of *E. coli* (46%, 58%), *L. monocytogenes* (6%, 10%), *Salmonella spp.* (10%, 14%), *Staphylococcus aureus* (4%, 20%), *Campylobacter spp.* (4%, 2%), and *Pseudomonas spp.* (2%) in both frozen and raw meat samples. These pathogens pose significant health risks through the consumption of raw or undercooked

contaminated meat, especially given that most foodborne illnesses have an animal origin.

While this study reports a more negligible prevalence of *Pseudomonas aeruginosa* compared to other research, such as Qasim (2019), Poursina et al. (2022), and Mahato et al. (2022), who observed higher rates of isolation, it still highlights the public health concern associated with mutton consumption. Dong et al. (2022) reported even higher rates of *Pseudomonas aeruginosa* isolation from retail meat samples, where 53 strains were isolated from 150 samples (35.33%). Conversely, Rezaloo et al. (2022) found a lower prevalence, with only 29 out of 370 (7.83%) meat and meat product samples infected with *P. aeruginosa*.

Higher isolation rates observed in some studies may be due to inadequate hygiene practices, extended exposure of meat to environmental contaminants, and differences in sampling methods, such as analyzing fresh versus frozen meats. In contrast, lower isolation rates may reflect stricter food safety regulations, enhanced sanitation during processing and retailing, or variations in laboratory detection techniques. Despite the lower prevalence found in our study, *P. aeruginosa* remains a significant public health concern due to its potential to cause infections and its increasing resistance to antibiotics. Therefore, even at lower isolation rates, ongoing surveillance and strengthened food safety measures are crucial to reducing the risks associated with foodborne pathogens like *P. aeruginosa*.

Mutton is a common meat source, and its consumption has increased worldwide because of its nutritional benefits over other red meats; this result may indicate that mutton meat is considered a public health problem (Mahmoud et al., 2022). Foodborne diseases are a major public health concern, causing widespread illness, hospitalizations, and in severe cases, death. The detection of *P. aeruginosa* in mutton meat highlights the urgent need to strengthen food safety standards in Iraq. To reduce the risk of contamination, food producers, processors, and retailers must take responsibility for ensuring that proper food safety protocols are consistently followed. In addition, educating consumers about safe food handling and storage practices is crucial to preventing contamination at the household level.

Antimicrobial Susceptibility Profiling of *Pseudomonas aeruginosa*:

The high percentage of antibiotic-resistant *Pseudomonas aeruginosa* isolates from mutton meat highlights a growing threat to both food safety and public health. The study identified that highly efficacious treatments against *Pseudomonas aeruginosa* include Colistin sulfate, Gentamycin, Amikacin, and Imipenem. There is also higher resistance to Ampicillin, Ceftazidime, Ofloxacin, Ticarcillin-Clevulanic acid, and Aztreonam and intermediate resistance to Cefepime, Ciprofloxacin, and Levofloxacin (**Table 3**).

These findings contrast with those of Bernie et al. (2017), who isolated *Pseudomonas aeruginosa* from bovine and fish meat, reporting high resistance rates for Aztreonam (98.4%), Ticarcillin + Clavulanic acid (51.4%), Ticarcillin (50.4%), and Piperacillin (31.4%). While the study reported lower resistance to Imipenem (7.2%), it is consistent with the current study in terms of Colistin resistance (4.5%)., and agreed with the current study by the percentages of Colistin (4.5%). The mechanisms underlying widespread antibiotic resistance involve various strategies, including the production of β-

lactamases, alterations in antibiotic target sites, efflux pumps, and biofilm formation. These factors contribute to the persistence of resistant strains in food products, posing significant risks to human health (Khudair and Mahmood, 2021).

In the present study, high resistance rates were observed for Aztreonam, Ceftazidime, and Levofloxacin, surpassing previous findings with resistance rates of 47%, 68%, and 33%, respectively (Khudair and Mahmood, 2021). Furthermore, the widespread resistance emphasizes the critical need for antibiotic stewardship in the agriculture and food production sectors. The contamination of meat products with such resistant strains can pose serious health risks, especially in regions with inadequate regulatory measures. *Pseudomonas aeruginosa* strains exhibited resistance to commonly used antibiotics, such as β-lactams, monobactam, and fluoroquinolones. The results agree with Mahmood., 2022. who found that the excessive use of antibiotics in livestock farming is a critical factor driving this resistance.

Poor hygiene practices during slaughter, processing, and distribution contribute significantly to the contamination of meat with antibiotic-resistant bacteria. Additionally, the misuse or improper use of antibiotics further exacerbates the spread of resistant strains. The study highlights the urgent need for improved food safety practices and stronger antibiotic stewardship to address and reduce the public health risks associated with antibiotic-resistant bacteria.

PCR technique

Detected of the Azurin gene, Phylogenetic Analysis and Tree Construction

The high percentage of the presence of the Azurin gene in *Pseudomonas aeruginosa* isolated from imported mutton meat may be due to the rich protein content that contains the most nutrients and materials that provide a good medium for bacterial growth. The percentage of azurin gene (84.61%) from imported mutton meat (Figure 4). This result agrees with (Sereena and Sebastian., 2016), who isolated the azurin gene from 80% of the infections of environmental sources. Another study (Barzelighi et al., 2019) isolated this gene from all samples (100%).

The Azurin gene in *Pseudomonas aeruginosa* plays a crucial role in enhancing the bacterium's virulence, survival, and resistance. It contributes to biofilm formation, adhesion, and the secretion of toxins, making *Pseudomonas aeruginosa* more pathogenic. Furthermore, strains of *Pseudomonas aeruginosa* that carry the Azurin gene have been associated with increased antibiotic resistance, which complicates treatment options and contributes to the problem of multidrug resistance (MDR). This situation raises significant concerns for both clinical infections and food safety, emphasizing the need for effective surveillance and management strategies to address its spread and impact on public health.

Results of azirine gene sequencing and phylogenetic data analysis showed that the Iraqi isolate of *Pseudomonas aeruginosa* from a mutton meat sample is 100% identical to the corresponding region in the *Pseudomonas aeruginosa* strain PAAK088 deposited in the NCBI database. This may be due to the contaminated meat during handling, transporting, and storage. Therefore, facilities should improve sanitation and enforce stricter food safety regulations.

The complete identity between the Iraqi isolate and strain PAAK088 suggests a close

genetic relationship, indicating that the Iraqi isolate shares a significant portion of its genetic material with this particular strain. This high similarity underscores the potential for horizontal gene transfer and the spread of resistant and virulent *Pseudomonas aeruginosa* strains between different regions or across geographical boundaries. Additionally, the persistence of such strains in food supplies, particularly in meat products, emphasizes the need for enhanced monitoring and biosecurity measures to prevent the spread of these resistant and pathogenic bacteria. The results underline the importance of implementing stringent food safety practices and antimicrobial stewardship programs to mitigate the risks associated with resistant *Pseudomonas aeruginosa* in the food supply chain.

CONCLUSION

The detection of multidrug-resistant *Pseudomonas aeruginosa* in mutton meat from Baghdad underscores a significant public health threat. This highlights the urgent need for enhanced food safety measures, including stricter hygiene practices, regular monitoring, and improved antimicrobial management in livestock. Public awareness and the prevention of antibiotic misuse in the food industry are also essential to combat resistance. Future efforts should focus on fostering collaboration between public health authorities and the meat industry to address these challenges effectively. Through comprehensive strategies, it is possible to reduce the risks posed by *P. aeruginosa* and ensure safer food for consumers.

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Conflict of interest:

The authors declare no conflict of interest.

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