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Antibiotics resistance patterns of *Pseudomonas aeruginosa* isolated from meat at Mosul city retails

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Abstract

Psychrotrophic bacteria are one of the significant microbes in chilled meat, pseudomonas is the most predominant member of this group. Pseudomonas could survive in various ecological niches. In the current study, we detect the prevalence of *Pseudomonas aeruginosa* in meat at Mosul city retails and their antibiotics resistance profile. One hundred fifty samples from beef, mutton and chicken meat were collected aseptically and cultivated. *P. aeruginosa* isolates were phenotypically and genetically recognized, and their antimicrobial activity was carried out for cultured isolates. Our findings revealed that 21 (14%) of meat samples were *P. aeruginosa* positive with a high significant recovery rate in chicken meat 11 (22%) and beef 7 (14%) compared to mutton 3 (6%). The antimicrobial resistance level of *P. aeruginosa* were 100% for amoxicillin, and 66% for aztreonam. The susceptibilities were 95% for tobramycin, 90% for levofloxacin, 90% for ciprofloxacin, 90% for gentamicin, 76% for piperacillin, and 57% for meropenem. In conclusion, *P. aeruginosa* is likely to be more common in meat, especially in chicken. Therefore, good hygienic practices should be applied to handle and preserve meat under suitable conditions to extend its shelf life, ensure meat safety, and conserve consumer health.

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Introduction

Meat quality is a multifaceted topic influenced by internal and external animal variables. The consumer recognizes quality through good sensory attributes. To avoid economic losses, the producer must meet organoleptic conditions; and ensure that the product remains microbiologically safe during storage (1). Meat spoiling is caused by a range of factors, including oxidation, enzymatic autolysis, and microbiological growth, which vary based on husbandry practices, animal species, ages at slaughtering, handling, processing, and preservation method. Microbial spoilage leads to pH alteration, degradation, and slime formation, reflecting off odor and loss of bloom (2,3). Pseudomonas is a significant gram-negative bacterium that spread widely in food, water, soil, and the environment due to their simple nutritional requirements. In addition, it has been related to human and animal illness and a high mortality rate in broiler chickens (4), it is an excellent concern in newly hatched chicks (5). It also has a high distribution within the food ecosystem (6). *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas fragi* are the most representative species of the genus in animal products, Pseudomonas has a significant value in meat and milk because of its ability to produce several lipolytic and proteolytic enzymes and develop a biofilm that impacts food quality (7-9). *P. aeruginosa* is an opportunistic pathogen associated with meat spoilage and reduces the shelf life of meat and meat products (10-12). It is associated with severe infections, especially in immunocompromised individuals (13,14). A single flagellum and multiple cell surface pili offer bacterial

movement and adhesion characteristics, allowing pseudomonas species to colonize surfaces, especially under cold and aerobic conditions (15,16). *P. aeruginosa* causes many infections that do not respond to treatment and are life threatening due to their high multi-drug resistance to antibiotics through their lower outer membrane permeability (17,18). The production of antibiotic-inactivating enzymes and the expression of efflux-pumps that expel the antibiotics out of the cell propagate antimicrobial resistance genes across the food chain, all of them increasing the resistance gene pool and posing a risk to the public health (19,20). Clinical isolates of *P. aeruginosa* possess a multidrug-resistance than the food and environmental isolates. Antimicrobial resistance of a prospective disease to a wide range of antibiotics could signal a severe problem that affects public health (21,22). Because there has been no previous research on the antimicrobial activity of *P. aeruginosa* isolated from meat, the current study was designed to evaluate the prevalence of *P. aeruginosa* in meat displayed in Mosul city retails and assess its antibiotic resistance patterns.

Materials and methods

Ethical approval

The scientific committee of the veterinary public health department approved this work on the twelfth session at 20/June/2021.

Samples

The study was based in Mosul city, and meat samples were taken from different markets and butcher's shops. One hundred fifty meat samples from beef, mutton, and chicken each 50 were collected randomly and under sterile conditions the samples were transported as soon as possible to the College of Veterinary medicine, Veterinary Public Health Laboratory, for further microbiological analysis.

Isolation and identifications

Meat samples were homogenized with nutrient broth under sterile conditions for enrichment. Additionally, cetrimide agar medium (Neogen, USA) was used to isolate Pseudomonas as a selective medium, Plates were inoculated using the streaked plate method and incubated aerobically at 37°C for 24 hours, and the pure culture of *P. aeruginosa* was exposed to ultra-violet light to detect the fluorescence ability. Detection of *P. aeruginosa* isolates was done microscopically based on using a light microscope to observe Gram staining, confirmed by biochemical tests (23,24). Molecular detection of *P. aeruginosa* were done using the *rpoB* gene to confirm the isolates (25).

Antibiotic susceptibility test

To assess the susceptibility of *P. aeruginosa* isolates to antibiotics, the standard Kirby-Bauer disk diffusion method was used according to Jorgensen and Turnidge (26). The antibiotics discs (Bioanalyse, Turkey) used against pseudomonas isolates including levofloxacin (LEV 5 µg), ciprofloxacin (CIP 5 µg), amoxicillin/clavulanic acid (AMC 30 µg), piperacillin (PRL 100 µg), aztreonam (ATM 30 µg), meropenem (MEM 10 µg), tobramycin (TOB 10 µg), gentamicin (CN 10 µg). The suspension of isolates equivalent to 0.5 McFarland opacity standard was prepared and inoculated on Mueller-Hinton agar plates using a sterile swab. Then, the discs were placed aseptically on the inoculated surface of the media and incubated for 24 hours at 37ºC. After the incubation period, the diameter of the inhibition zone to each disc was measured using a digital caliper (Ingco, China). The results were translated to Susceptible, Intermediate, and Resistant categories by comparing inhibition zone diameters according to manufacturer instructions.

Statistical analysis

Statistical analysis of the observed data was done with a Chi-square test using SPSS software version 22.

Results

Depending on molecular detection of *P. aeruginosa* according to the presence of the *rpoB* gene by conventional PCR, out of 150 meat samples collected from Mosul city retails, twenty-one isolates of *P. aeruginosa* were positive with a total recovery rate pf 14% (Table 1). The results revealed significant differences between the recovery rate of *P. aeruginosa* from beef and chicken meat compared to mutton at $P<0.05$. Additionally, typical colonies of Pseudomonas on cetrimide agar medium are shown revealing characteristics of shiny, smooth, convex greenish to yellow colonies (Figure 1a). The exposure of colonies on cetrimide agar to UV light exhibits blue color as in (Figure 1b).

Table 1: The prevalence of *Pseudomonas aeruginosa* in different types of meat

Type of meat sample	No. samples	No. +ve *Pseudomonas aeruginosa*	Recovery rate (%)
Beef	50	7	14
Mutton	50	3	6
Chicken	50	11	22
Total	150	21	14

Among the 21 isolates of *P. aeruginosa* from different types of meat, the antimicrobial resistance profile revealed high resistance of isolates to amoxicillin at 100%, aztreonam at 66%, while meropenem was recorded at 14%. In contrast, *P. aeruginosa* isolates were susceptible to tobramycin 100%, levofloxacin 90%, ciprofloxacin 90%, gentamicin 90%,

followed by piperacillin 76% and meropenem 57%. At various levels, some strains showed a multi-drug resistance to amoxicillin, aztreonam, and meropenem (Figure 2).

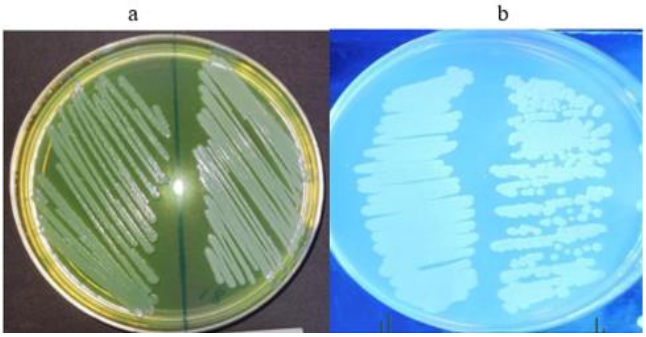

Figure 1: (a) growth of *P. aeruginosa* on cetrimide agar, (b) fluorescence activity under UV-light.

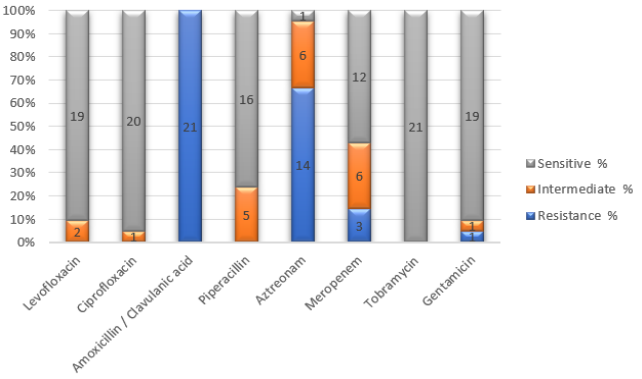

Figure 2: Antibiotics resistance patterns of *P. aeruginosa* isolated from meat

Discussion

Pseudomonas is a potent indicator of food spoilage, especially in meat and meat products leading to putrefaction under aerobic conditions (27). Meat processing can be the primary source of contamination in fresh meat and meat products that are not exposed to heat during preparation (28). Our results exhibited 14% *P. aeruginosa* recovery rate in meat displayed at Mosul city retails, which may be related to defects in storage temperature. These results agree with the recovery rate of *P. aeruginosa* reported by Hemmat (29) in frozen imported meat. In contrast the prevalence of *P. aeruginosa* in bovine meat was 47. 8% in Abidjan (30). The differences in the prevalence of *P. aeruginosa* in beef, mutton, and chicken meat may be related to the initial contamination level. Intrinsic properties of meat despite the variation in the recovery rate of *P. aeruginosa* from animal sources, the presence of these microbiotas in meat reflects the weakness of hygienic measurement applied in meat production and marketing, which negatively impacts meat quality and accelerates spoilage (31,32). The low isolation rate of *P. aeruginosa* in mutton may be due to the elevation of other microbiota such as *p. fluorescens* and enterobacteriaceae through competition. Also, a higher intramuscular fat content of mutton reduces the growth of spoilage bacteria, especially if it was preserved under vacuum packaging (33,34). The antibiotics resistance patterns of Pseudomonas varied according to phylogeny. The *P. aeruginosa* strains isolated from different types of meat were sensitive to aminoglycosides, fluoroquinolones, penicillin and carbapenems with total isolates resistance to amoxicillin and high resistance to aztreonam. These results agree with (35,36) who conducted that the resistance to beta-lactams was related to the presence of plasmid-mediated extended-spectrum beta-lactamases. Another study conducted by Murphy *et al.* (37) on frozen meat accepts our findings. Moreover, 29% of isolates show intermediate resistance to meropenem, this high percentage of resistance may be related to the indiscriminate use of antibiotics by veterinarians in farm animals as treatment or as growth promoters and can be transmitted to human beings (38,39). The most effective antibiotics against *P. aeruginosa* were tobramycin, levofloxacin, ciprofloxacin and gentamicin similar to pseudomonas resistance recorded in poultry meat by Kousar *et al.* (40). Also, poultry farms spread multi-drug resistance in their environment (41,42). Both Piperacillin and Meropenem are the second most effective antibiotics against *P. aeruginosa* from meat. Our results were approved by Glen and Lamont (43) who reported ciprofloxacin as a potent drug against Pseudomonas. Due to the excessive use of antibiotics in livestock farms, a severe public health hazard is developed. Therefore, possible strategies are essential for enhancing the effectiveness of antibiotics, especially the beta-lactam. in treating *P. aeruginosa* infections (44,45). Finally, proper handling of raw products, adequate cooking of meat, and proper disposal of waste, can minimize the antibiotics resistance of these bacteria, which may reflect the misuse of antibiotics in animals.

Conclusion

Antimicrobial resistance is a significant challenge to food safety and consumer health. The antibiotic resistance of *P. aeruginosa* may generate a burden and limit the choice of antibiotics due to resistance development.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest.

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أنماط المقاومة للمضادات الحيوية في الزوائف الزنجارية المعزولة من اللحوم في أسواق مدينة الموصل

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الخلاصة

تعد الجراثيم المحبة لدرجات حرارة التبريد أحد الأحياء المجهرية المهمة في اللحوم المبردة وتعتبر الزوائف أحد الأنواع السائدة ضمن هذه المجموعة، وتتمكن الزوائف من العيش في ظروف بيئية متعددة. تم الكشف في الدراسة الحالية عن مدى تواجد جراثيم الزوائف الزنجارية في اللحوم من أسواق مدينة الموصل وحساسيتها للمضادات الحيوية. جمعت ١٥٠ عينة من لحوم الأبقار والأغنام والدواجن بطريقة معقمة وزرعت للكشف على عزلات الزوائف الزنجارية والتي تم تشخيصها مظهريا وجينيا وتحديد فعاليتها للمضادات الحيوية. أظهرت النتائج تواجد ٢١ عزلة موجبة من جراثيم الزوائف الزنجارية وبنسبة عزل كلية بلغت ١٤% وكانت نسبة العزل مرتفعة معنويا في لحوم الدواجن ١١ (٢٢%) وفي لحوم الأبقار ٧ (١٤%) مقارنة بلحوم الأغنام ٣ (٦%). وكانت مقاومة جراثيم الزوائف الزنجارية للمضادات الحيوية بنسبة ١٠٠ % للاموكسيسيلين و٦٦% للازترونام وبمدى حساسية ٩٥%للتوبرومايسين و٩٠% لكل من الليفوفلوكساسين والسيبروفلوكساسين والجنتاميسين بينما كانت ٧٦% للبيبراسيلين و٥٧% للميروبينيم. نستنتج أن جراثيم الزوائف الزنجارية قد تكون أكثر الأنواع شيوعا في لحوم الدواجن لذا يفضل تطبيق الشروط الصحية المناسبة أثناء تداول اللحوم وحفظها بظروف مناسبة لإطالة مدة حفظها وضمان سلامة اللحوم حفاظا على صحة المستهلك.

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