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Molecular Identification of *Pseudomonas aeruginosa* in meat at Mosul city retails using PCR technique

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Abstract

Pseudomonas has been recognized as a unique meat spoiling organism. The proliferation of these spoilage organisms might influence the organoleptic meat quality. Therefore, the current investigation is being carried out to detect pseudomonas associated with meat displayed in Mosul city retails. A total of 150 meat samples of beef, mutton and chicken meat (50 of each) were collected. Molecular identification of *pseudomonas aeruginosa* in meat is performed by targeting the *16S rRNA* gene and *rpoB* gene. Fifty-three isolates of pseudomonas species were obtained from all types of meat (35.33%), including 23 (46 %) for beef meat,11 (22%) for mutton and 19 (38%) for chicken meat. Enumeration of pseudomonas species in beef and mutton were $1.47*10^4$, $1.92*10^4$ CFU/g, respectively, while counts were $21.3*10^4$ CFU/g in chicken meat. Polymerase chain reaction results revealed the presence of *16SrRNA* gene in all tested isolates 53/53 (100%). *pseudomonas aeruginosa* was isolated at (39.62%) from meat samples according to the detection of the *rpoB* gene. In conclusion, the prevalence of pseudomonas in meat at Mosul city retails negatively impacted meat quality and consumer confidence. Also, the PCR approach aids the rapid detection of pseudomonas as spoilage organisms in meat to reduce financial loss. Therefore, hygienic measurements should be applied to reduce meat spoilage and conserve consumer health during meat production and preservation.

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Introduction

Meat is a source of animal proteins for human being nutrition, constituting a suitable medium for the growth of many pathogens (1). It can be contaminated during slaughtering and dressing, initially dressed carcasses may be exposed to contamination by some microorganisms which may survive during meat processing and storage (2,3). The shelf life of meat is extended under good hygienic practices during storage. After chilling psychrotroph may gradually appear, the most common one is pseudomonas which is dominant in chilled meat stored under aerobic conditions (4). Before consuming meat, the proliferation of pseudomonas affects the organoleptic properties of meat, leading to the loss of the product (5). Pseudomonas strains are specific spoilage microbial species found in meat, chicken, and fish. They are recognized through the nitrogenous components that produce the volatile compounds such as ketones, aldehydes, and esters) which causes the off-flavor to appear at the spoilage point (6,7). Pseudomonas can be utilized glucose as a substrate, when the glucose is exhausted, most amino acids can be used for growth, leading to meat deterioration and becoming unacceptable to consumers (8). The significant risk of pseudomonas to consumer health is its high resistance to antimicrobials due to the impermeability of the outer membrane lipoprotein (9). Currently, molecular identification of microorganisms is widely practiced in food microbiology (10,11). Molecular detection of pseudomonas species diversity in meat is vital to provide suitable strategies for monitoring spoilage related

microbiota in meat to retard its growth and extend its shelf life of meat (12). *16S rRNA* gene sequence analysis is used to detect pseudomonas (13). *rpoB* gene is also used as a target to identify the presence of *p. aeruginosa* as a contaminant in meat (14). The current study has focused evaluating meat contamination in Mosul city retails with psychrotrophic *P. aeruginosa*.

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

One hundred fifty meat samples from Mosul city retail shops were collected randomly and distributed into 50 samples each of beef meat, mutton meat, and chicken meat. Meat samples were placed in the icebox and transported to the Veterinary Public Health Laboratory, College of Veterinary Medicine, University of Mosul.

Isolation and counting of pseudomonas

25 g of meat sample were added to 225 ml sterile peptone water, homogenized, and then, serial decimal dilutions were prepared; 0.1 ml of each dilution was spread on Pseudomonas cetrimide agar (PCA) (Neogen, USA). Plates were incubated at 25°C for 48 hours (15). Phenotypical characteristics were examined.

Identification of bacterial isolates

Pseudomonas species isolates were identified microscopically based on Gram staining, under a light microscope, and biochemical tests including the catalase test, oxidase test, production of Pyoverdine and growth at 42°C and 5°C were depended to confirm the identification of *p. aeruginosa* (16).

DNA extraction of bacterial isolates

Positive colonies of pseudomonad purified on nutrient agar were subjected to DNA extraction. Extraction of DNA was done according to the protocol of the supplying company (Jena Bioscience, Germany) suspending the selected colonies in 1.5 ml eppendrof microcentrifuge for cell lysis, centrifuge at 15,000x g for 1 minute. The supernatant was discarded, the bacterial sediment was taken, and the pellet was resuspended in 300 µl of cell lysis solution. The tubes were placed in the vortex mixer for 5 minutes and then centrifugated at 10,000 X g for one minute. The supernatant was transferred to 1.5 ml microtube containing 300 µl of absolute Isopropanol for DNA precipitation, mixed gently by inverting for 1 minute, and centrifugated at 15,000x g for 1min. The supernatant was discarded. The DNA pellets were washed by adding 500 µl of washing buffer and centrifuged at 15000× g for 1 minute, followed by supernatant elimination and drying in air at room temperature; 100 µl of Hydration Solution was added to dried DNA followed by incubating at 65°C for 60 min. to accelerate hydration. Extracted DNA was stored at -20℃ for further use.

Amplification of *16S rRNA* gene and *rpoB* gene

The existence of *16S rRNA* gene and *rpoB* gene were investigated to identify pseudomonas species. using polymerase chain reaction assay. Amplification of the *16S rRNA* gene and *rpoB* gene were done using the forward and reverse primers according to Franzetti and Scarpellini (17), the *16S rRNA* primer used in the present study with a molecular weight of 1351 bp F:5' AGAGTTTGATCCTGGCTCAG'3; R: 5' CTACGGCTACCTTGTTACGA'3, while the *rpoB* gene primer is 750 bp F: 5' CAGTTCATGGACCAGAACAACCCG'3; R: 5' ACGCTGGTTGATGCAGGTGTTC'3 . All components of the PCR reaction were placed in the PCR tube (Citotest, China). The total volume of a PCR reaction was 25 µl. contained 2 µl of DNA, 1 µl of each primer, 12.5 µl of Master Mix 2X, and 8.5 µl of nuclease free water. The amplification reactions were performed in a thermal cycler (BioRad, T100, USA) according to the instructions of the manufactured company included initial denaturation of 10 min at 94°C followed by 35 cycles 45 sec at 94°C for denaturation, 45 sec at 55°C and 58°C for annealing to each of *16SrRNA* and *rpoB* gene respectively, then extension of 1min at 72°C and final extension of 10 min at 72°C.The reaction was cooled at 4 °C. Separation of PCR products by electrophoresis employed 1.2% agarose gel (Promega, USA) containing prime safe dye (GeNet Bio, Korea). 5 µl of each PCR product were loaded on the wells of agarose gel. Electrophoresed in 70 volts 300 mA for 55 minutes, A volume of 4 µl of DNA ladder, 100 bp (GeNet Bio, Korea) was used as standard. The gel was captured using the Gel doc EZ system (Bio-Rad-USA) to distinguish the specific bands.

Results

Out of one hundred fifty meat samples, fifty-three Pseudomonas strains recovered at 35.33% using pseudomonas cetrimide agar distributed at 46% for beef meat, 22% for mutton, and 38% for chicken meat, the count of Pseudomonad species were $1.47*10^4$, $1.92*10^4$ and $21.3*10^4$ CFU/g for beef, mutton, and chicken meat respectively (Table 1). The PCR results indicated that all pseudomonas isolates were positive for *16SrRNA gene* producing product size1351 bp (Figure 1), while 21 *P. aeruginosa* were positive from the different types of meat at 39.62% using *rpoB gene* producing product size 750 bp (Figure 2).

Table 1: The prevalence of Pseudomonas species in examined meat samples

Type of meat sample	No. samples	No. *pseudomonas spp.*	% Isolation	*Pseudomonas spp.* CFU/g
Beef meat	50	23	(23/50)46	$1.47*10^4$
Mutton	50	11	(11/50) 22	$1.92*10^4$
Chicken meat	50	19	(19/50) 38	$21.3*10^4$
Total	150	53	(53/150) 35.33	

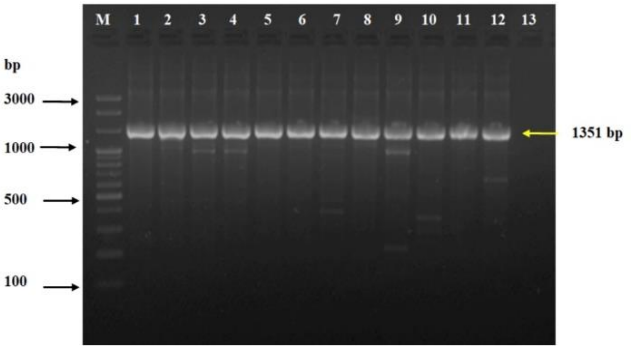

Figure 1: Electrophoretic profile illustrates Lanes M, DNA marker; lane 1-12, *16SrRNA* gene of *pseudomonas* at 1351 bp product size, lane 13 negative control.

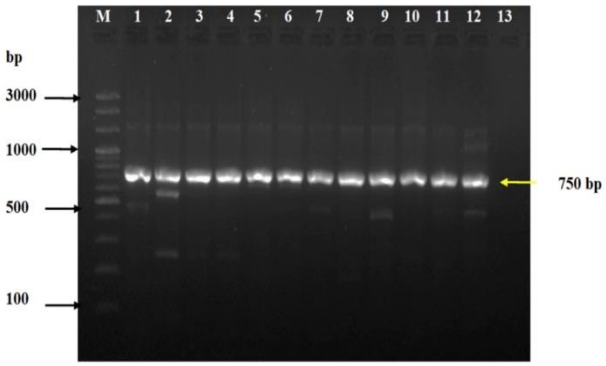

Figure 2: Electrophoretic profile illustrates Lanes M, DNA marker; lane 1-12, *rpoB* gene of *p. aeruginosa* at 750 bp product size, lane 13 negative control.

Discussion

Pseudomonads are dominant organisms in meat maintained under aerobic conditions at chilled temperatures due to their distinct glucose metabolism, Pseudomonas utilize glucose as a substrate till glucose is exhausted, followed by degradation of amino acids, prevalence in meat has an environmental origin (18,19). The results revealed an essential contribution of pseudomonas species in meat. In our study the isolation rates of *p. aeruginosa* from chicken meat are higher than the incidence rate of *p. aeruginosa* recorded in meat in Samsun province obtained by Sırıken *et al*. (20) and less than the isolation rate indicated by Abd El-Aziz (21). The prevalence of *p. aeruginosa* in chicken meat could be linked to the contaminated eggs and hatcheries in conditions conducive to microbiota growth accompanied by economic problems (22,23). Our finding obtained from the isolation of pseudomonas from beef meat 46% are disagreements with the results of Hemmat *et al.* (24) and may be owing to metabolic variation among pseudomonas species in meat which affect the dominance of some Pseudomonas species in meat, such as *P. fragi* over *P. lundensis* and *P. fluorescens* due to its ability to metabolize creatine and creatinine under aerobic conditions (25). The prevalence of *p. aeruginosa* in beef meat can be associated with the low susceptibility to a various antimicrobial (26). The average count of pseudomonas in chicken meat is closely related to that found by Keskin and Ekmekçi (27) in chicken meat in Izmir. To overcome difficulties with culture methods using specific primer such as the *rpoB* gene by PCR revealed the presence of *P. aeruginosa* in meat (28). Our findings highlight the importance of understanding the most common pseudomonas clones in meat displayed in retail supermarkets and butcher shops and their risk to consumer health. Further research is needed to improve preservation against spoilage bacteria.

Conclusion

Meat is a possible source of the potential hazard for transmitting various pathogens to the consumer, pseudomonas is a specific spoilage organism. Our results show that the prevalence of *pseudomonas aeruginosa* in meats may influence the keeping quality. Good hygienic measures are required to restrict the presence of such pathogens and extend the shelf life of meat to ensure meat safety.

Acknowledgments

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Conflict of interest

The authors have approved the manuscript and declare no conflict of interest.

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الكشف الجزيئي عن الزوائف الزنجارية في اللحوم في أسواق مدينة الموصل باستخدام تقنية تفاعل البلمرة المتسلسل

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الخلاصة

تميزت الزوائف بكونها احدى الكائنات المجهرية المسببة لفساد اللحوم وتكاثرها في الغذاء قد يؤثر على الصفات الحسية للحوم وجودتها لذا هدفت الدراسة الحالية الى الكشف عن جراثيم الزوائف المقترن تواجدها في اللحوم المعروضة في الأسواق في مدينة الموصل. جمعت ١٥٠ عينة لحم من لحوم الأبقار والأغنام والدواجن بواقع ٥٠ عينة لكل نوع منها. استهدفت الدراسة التشخيص الجزيئي لجراثيم الزوائف الزنجارية في اللحوم بالاعتماد على وجود الجينات *16SrRNA* و *rpoB*. تم الحصول على ٥٣ عزلة من جراثيم الزوائف من كل أنواع اللحوم وبنسبة ٣٥,٣٣٪ موزعة على ٢٣ (٤٦٪) من لحوم الأبقار و ١١(٢٢٪)

من لحوم الأغنام و ١٩ (٣٨٪) من لحوم الدواجن. كان العد الكلي للزوائف في لحوم الابقار والاغنام ١٠*١,٤٧ ٤ و ١٠*١,٩٢ ٤ وحدة تكوين المستعمرة / غرام على التوالي بينما أظهر العد الكلي للزوائف في لحوم الدواجن ١٠×٢١,٣ ٤وحدة تكوين المستعمرة /غرام. أظهرت نتائج تفاعل البلمرة المتسلسل وجود جين *16S rRNA* في كل العزلات الموجبة للزوائف (٥٣/٥٣) وبنسبة ١٠٠٪. عزلت الزوائف الزنجارية بنسبة (٣٩,٦٢٪) من اللحوم بالاعتماد على وجود الجين *rpoB*. نستنتج من هذه الدراسة مدى تواجد جراثيم الزوائف في اللحوم المعروضة في أسواق مدينة الموصل والذي يؤثر وبشكل سلبي على نوعية اللحوم وجودتها وعلى ثقة المستهلكين بهذه الأنواع من اللحوم كما أن اعتماد تقنية تفاعل البلمرة المتسلسل يساهم في سرعة تشخيص الزوائف المسببة لفساد اللحوم ويقلل من الخسائر الاقتصادية المترتبة على ذلك لذا يتوجب اتباع الشروط الصحية عند إنتاج اللحوم وحفظها للحد من فساد اللحوم والحفاظ على صحة المستهلك.