Detection of some virulence genes of *Pseudomonas aeruginosa* isolated from meat at Mosul city

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Abstract

Meat is a rich source of protein for humans. Pseudomonas is one of the meat spoilage microorganisms, as it is highly pathogenic and affects the health of consumers and meat handlers. The current study detected the presence of some virulence genes in *Pseudomonas aeruginosa* strains isolated from meat in Mosul city, including *ToxA*, *ExoS*, *OprL*, and *PlcH* virulence genes in 21 isolates of *P. aeruginosa* using PCR. Results revealed the presence of *ToxA*, *OprL*, and *PlcH* genes at 57.14%, 38.09%, and 71.42%, respectively, while all the isolates of *P. aeruginosa* were negative for the presence of the *ExoS* gene. The prevalence of *ToxA* and *PLcH* genes was higher in beef meat compared to mutton and chicken meat, while the *OprL* gene was not detected in mutton. These results indicated that some strains of *P. aeruginosa* are pathogenic to meat handlers and consumers; following food safety practices must be applied in the meat production chain to prevent meat contamination with pathogenic bacteria.

Introduction

In Mosul, people eat a variety of meats displayed in markets, such as beef, mutton, and chicken. Improper hygienic conditions in handling and preserving meats predisposed them to spoilage because meat is a suitable medium to growth of different types of microorganisms (1,2). Proliferations of these bacterial groups lead to meat deterioration (3,4) especially psychrotrophic bacteria which can arise in meats stored aerobically at low temperature (5-7). *Pseudomonas aeruginosa* is one of the most common specific spoilage microorganisms in meat and meat products which make them unpalatable as a result of discoloration, off-odor, off-flavor, and slime production (8-10). *Pseudomonas aeruginosa* described as an opportunistic pathogen associated with health hazards (11-13). Along with skin infections, markedly contributed to a wide range of infections in many organs, including the respiratory, urinary, and gastrointestinal tracts due the presence of cell mediated virulence factors like flagella, pilli, lipopolysaccharides, exoproteases, exotoxins and phospholipase C which are frequently implicated in bacterial motility and colonization (14,15). In addition, *P. aeruginosa* has the ability to form biofilms which increased its resistance to antimicrobial agents (16,17). Exotoxin A is one of these virulence elements that is crucial for tissue lysis and bacterial invasion. It employs a pilus-like apparatus to release proteins into the extracellular environment, including lipase, phospholipase, alkaline phosphatase, and protease (18,19). Lecithin and lipids that contribute to tissue invasion are destroyed by the hemolysin phospholipase H (plcH) enzymes. Additionally, *P. aeruginosa* produces elastase B (lasB), a key player in the acute infection, and exoenzyme S (*ExoS*), a cytotoxin that causes harm to a variety of host cells. It's an ADP ribosyl transferase that is secreted by the type III secretion system straight into the cytoplasm of epithelial cells (20). *OprL*, gene is also used as a marker to rapid identification of virulent *P. aeruginosa* strains (21).

Available studies about the virulence factors of *P. aeruginosa* in meat are not sufficient. The current study aimed to detect some virulence factors genes in *P. aeruginosa* isolated from beef, mutton, and chicken meat displayed in Mosul city retails using PCR technique.

Materials and methods

Ethical approve

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

Samples

The study included twenty-one isolates of *P. aeruginosa* obtained from 150 samples of fresh beef, mutton and chicken meat displayed in Mosul city retails in our previous study, these isolates were distributed as 7 isolates from beef meat, 3 isolates from mutton, and 11 isolates from chicken meat, the isolates were previously confirmed using molecular detection depending on *rpoB* gene using polymerase chain reactions (22,23).

Isolation and identifications

P. aeruginosa isolates from meat was inoculated on brain heart infusion broth media (Himedia /Indian) and incubated at 37°C for 24hrs. Followed by inoculation of growing bacteria on cetrimide agar (Neogen/USA) then incubated overnight at 37°C to select the pure isolates of *P. aeruginosa* (24).

DNA extraction

Genomic DNA of *P. aeruginosa.* was extracted using bacterial DNA preparation kit (Gena bioscience/Germany) following the manufacturer's instructions (23).

Polymerase chain reaction (PCR)

Molecular detection of *P. aeruginosa* virulence genes was done using specific primers targeting some virulence genes including ToxA, *ExoS*, *OprL* and *PlcH*. Each virulence genes were amplified using specific primer sequences mentioned in table 1, and provided by (Macrogen/Korea). The PCR master mix reaction prepared according to kit instructions (GeNet Bio, Korea) in 20 µl total volume by adding 2 µl of purified genomic DNA, 10 of Taq Premix (2X) and 1 µl of each forward and reverse primer 10 pmol /µl then completing the PCR premix tube by PCR grade water into 20 µl and briefly mixed by. The reaction was performed in a thermocycler (BioRad, USA) for amplification by applied the following thermocycler conditions; initial denaturation temperature of 95°C for 5 min; followed by 35 cycles at denaturation 95°C for 45s, annealing for 45 s at 55°C for each *ToxA* and *OprL* gene while the annealing of *ExoS* and *PlcH* for 45s. at 60°C followed by extension 72°C for 1min and then final extension at 72°C for 10 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel (BioRad, USA), stained with 3 µl prime safe dye (GeNet Bio, Korea), 4 µl of DNA ladder, 100 bp (GeNet Bio, Korea) depended as a molecular weight standard and the gel viewed using Gel doc Ez system (BioRad, USA) to identify the specific bands.

Statistical analysis

Chi-square test ($\chi 2$) was used to compare the prevalence of virulence factor genes among the isolates of *P. aeruginosa* strains and meat type (beef, mutton, and chicken) using IBM/SPSS/Statistics/version22, USA. A value of $P<0.05$ was considered statistically significant.

Table 1: primers sequence for some *P. aeruginosa.* virulence genes

Primers	Primer's sequence	°C	Size (bp)	Genes	References
ExoS-F	GCGAGGTCAGCAGAGTATCG	60	118	*ExoS*	(25)
ExoS-R	TTCGGCGTCACTGTGGATGC				
Oprl-F	ATGGAAATGCTGAAATTCGGC	55	504	*oprL*	(26)
Oprl-R	CTTCTTCAGCTCGACGCGACG				
ToxA-F	GACAACGCCCTCAGCATCACCAGC	55	396	*ToxA*	(27)
ToxA-R	CGCTGGCCCATTCGCTCCAGCGCT				
PlcH-F	GAA GCC ATG GGC TAC TTC AA	60	307	*plcH*	(28)
plcH-R	AGA GTG ACG AGG AGC GGTAG				

Results

Results highlighted the prevalence of some virulence factors genes of *P. aeruginosa.* strains isolated from meat. The *ToxA* gene was detected in 57.14% (12/21) of isolates.

The *OprL* gene was detected in 38.09% (8/21) of isolates. Also, *PlcH* gene was present in 71.42% (15/21) of *P. aeruginosa.* isolates. While all the isolates were negative for the exotoxin encoding S (*ExoS*) gene. The differences between the *PlcH*, *OprL,* and *ToxA* genes were statistically

significant at P<0.05. The prevalence of *ToxA* and *PlcH* genes was higher in beef meat 33.33% to each of them compared to mutton and chickens' meat while the prevalence of *OprL* genes was absent in mutton (Figures 1-5).
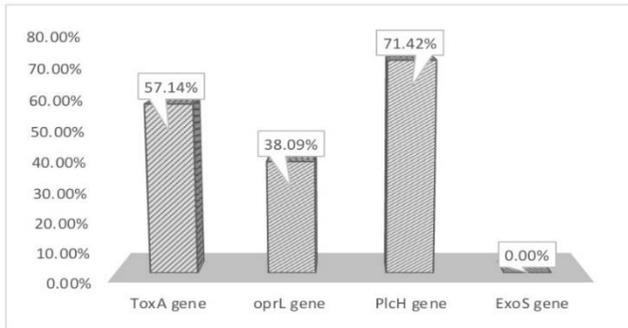

Figure 1: Prevalence of some virulence genes among *P. aeruginosa.* isolates from all types of meat.
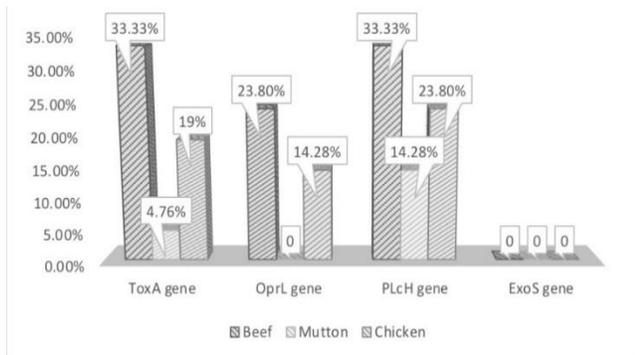

Figure 2: Prevalence of some virulence genes among *P. aeruginosa.* isolates from beef, mutton and chicken meat.
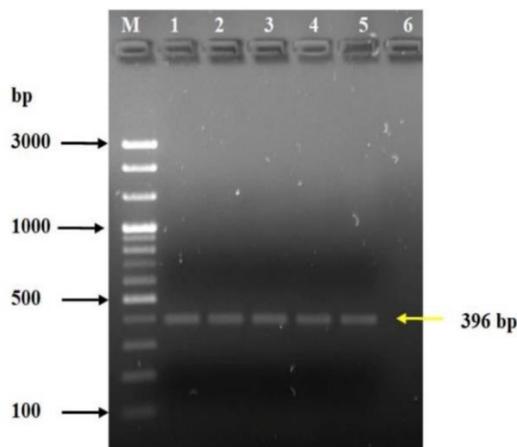

Figure 3: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-5, *ToxA* gene of *P. aeruginosa* at 396 bp product size, lane 6 negative control.
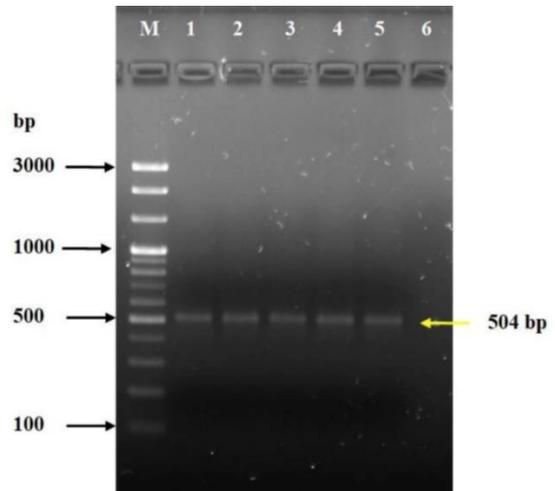

Figure 4: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-5, *OprL* gene of *P. aeruginosa* at 504 bp product size, lane 6 negative control**.**
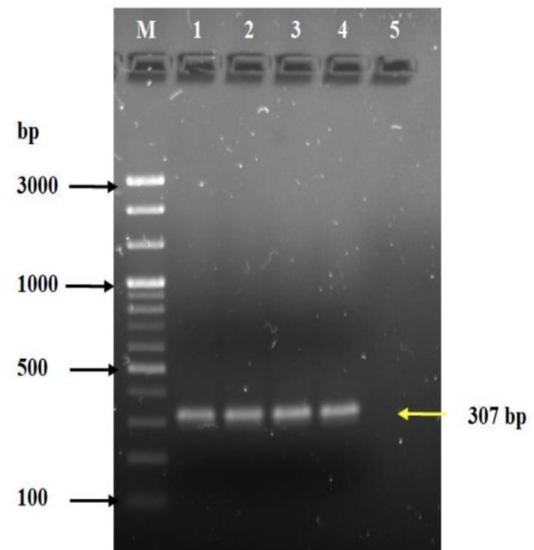

Figure 5: Electrophoretic profile illustrate Lanes M, DNA marker; lane 1-4, *PlcH* gene of *P. aeruginosa* at 307 bp product size, lane 5 negative control.

Discussion

P. aeruginosa from animal sources involved in many tissues destruction in human beings indicated their pathogenicity which is associated with the role of some virulence factors causing serious infections (29). Virulence factors of *P. aeruginosa* participate with an important role in the presence and growth of these microorganisms on the surfaces and invasion of tissues with the assistance of Pillis,

fimbria and polysaccharides which considered as a predisposing factor for infections (30,31). The prevalence of *ToxA* virulence genes in *P. aeruginosa* isolates from meat was relatively high while these virulence genes were absent in *P. aeruginosa* isolated from frozen meat (32). The prevalence of *oprL* genes in *P. aeruginosa* isolates from meat were 38.09% which disagreements with Tartor and El-Naenaeey (32) who referred to absence of these genes in frozen meat. Also, the prevalence of *PLcH* genes in *P. aeruginosa* isolates were 71.42% indicating that the isolates were capable of secreting phospholipase C and hemolytic exoenzyme which may involve in pulmonary infections (33) this percentage is relatively similar to the presence of *PlcH* genes in *pseudomonas* isolated from beef (34). The high prevalence of this gene indicated its importance in the pathogenicity of these microorganisms and its ability to induce infections with high potential risk to consumer (35,36) Meanwhile the distribution of *ExoS* genes in *P. aeruginosa* isolates from meat are negative these results were disagreed with Shahat *et al.* (37) who referred to the presence of these genes at 71.42% of *P. aeruginosa* isolates in broilers. The detection of some virulence factors of *P. aeruginosa* may be useful in early detections of these microorganisms in meats (38). The genetic variations in *P. aeruginosa* isolates from different animal sources play an important role in determination of their virulence according to source of isolates. Further studies are required to exhibit the association of *P. aeruginosa* virulence factors with public health especially those associated with serious respiratory and urinary infections.

Conclusion

Detection of some virulence factors genes in *P. aeruginosa* isolated from meat indicated the high level of pathogenicity of this microorganism which may affect the consumer health. The high prevalence of these virulence genes requires to apply hygienic conditions during the handling of meat and meat products as well as through their displaying in retails.

Acknowledgments

Conflict of interest

There are no conflicts of interest declared by the authors.

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الكشف عن بعض جينات الضراوة في الزوائف الزنجارية المعزولة من اللحوم في مدينة الموصل

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الخلاصة

عدت اللحوم من المصادر الغذائية الغنية بالبروتين وشخصت الزوائف الزنجارية كإحدى اهم الأحياء المجهرية المسببة لفساد اللحوم وتمتلك الزوائف إمراضية عالية قد تؤثر على صحة المستهلكين والمتداولين لهذه الأنواع من اللحوم. صممت الدراسة الحالية للكشف عن تواجد بعض عوامل الضراوة في عترات الزوائف الزنجارية المعزولة من لحوم الأبقار والأغنام والدجاج في مدينة الموصل وشملت الكشف عن كل من جينات الضراوة toxA, *ExoS, OprL and PlcH* في ٢١ عزلة من الزوائف الزنجارية باعتماد تقنية تفاعل البلمرة المتسلسل. أظهرت النتائج تواجد جين *toxA* و *OprL* و *PlcH* في عزلات الزوائف الزنجارية بنسبة ٥٧.١٤ و ٣٨.٠٩ و ٧١.٤% على التوالي بينما أعطت جميع العزلات نتيجة سالبة لتواجد جينات *ExoS*، وكانت مدى تواجد جينات *ToxA* و جينات *PlcH* أعلى في لحوم الأبقار مقارنة بلحوم الأغنام والدواجن بينما لم يشخص وجود جينات *OprL* في لحوم الأغنام. نستنتج من ذلك أن بعض عترات الزوائف الزنجارية قد تكون ممرضة لمتداولي ومستهلكي هذه الأنواع من اللحوم الأمر الذي يتطلب تطبيق الشروط الصحية خلال سلسلة إنتاج اللحوم وحفظها بظروف مناسبة لضمان وصولها للمستهلك آمنة وصحية.