eISSN 2255-0569

ORIGINAL

Virulence characters and oligotyping of *Pseudomonas aeruginosa* isolated from meat and assessment of the antimicrobial effects of *Zataria multiflora* against isolates

Caracteres de virulencia y oligotípicos de Pseudomonas aeruginosa aislada de la carne y evaluación de los efectos antimicrobianos de Zataria multiflora contra los aislados

Mohammadhosseini Sakhaei Shahreza[1], Nastaran Ghaderi Dehkordi[1], Maadh Fawzi Nassar[2], Raed Muslim Muhibes Al-Saedi[3]

1. Doctor Veterinary Medicine, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
2. Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3. Lecturer, Ph.D Organic Chemistry, Department of Biochemistry, Medical College, Missan University, Iraq

Corresponding author
Raed Muslim Muhibes Al-Saedi
E-mail: Raed_muslim7@gmail.com

Received: 2 - VI - 2022
Accepted: 12 - VI - 2022

doi: 10.3306/AJHS.2022.37.05.11

Abstract

Background: *Pseudomonas aeruginosa* is considered opportunistic pathogen responsible for some cases of food spoilage and probable foodborne diseases. The present survey was done to assess the prevalence, virulence characters, and oligotyping of *P. aeruginosa* strains isolated from meat samples and evaluate the antimicrobial effects of *Zataria multiflora* against isolates.

Methods: Two-hundred and seventy raw meat samples were collected and presence of *P. aeruginosa* was assessed using the culture. Isolates were subjected to sequencing and oligotyping and also PCR procedure to obtained the virulence characters. *Z. multiflora* aerial parts were used to prepare essential oil. Gas chromatography was used to determine chemical components. Disk diffusion was used to assess the antimicrobial effects of *Z. multiflora*. Minimum Inhibitory Concentration and Minimum Bacterial Concentration of *Z. multifl*ora was also assessed.

Results: Twelve out of 270 (4.44%) meat samples were contaminated with *P. aeruginosa*. Raw bovine meat had the highest *P. aeruginosa* prevalence (7.14%). ExoS (41.66%) was the most commonly detected virulence factors, while *algD* was the rarest (8.33%). Four oligotypes were detected in *P. aeruginosa* isolates. Oligo_7 (33.33%) had the highest distribution. Thymol (22.75%), carvacrol (15.81%), caryophyllene oxide (8.84%), and a-Pinene (7.73%) were the most commonly identified phytochemical compounds in the *Z. multiflora* essential oil. The dimeter of the growth inhibition zone of *P. aeruginosa* isolates treated with *Z. multiflora* (1%) essential oil was statistically higher than penicillin, gentamicin, ampicillin, and tetracycline ($P< 0.05$) and insignificantly lower than azithromycin ($P> 0.05$). The MIC and MBC of *P. aeruginosa* isolates treated with *Z. multiflora* essential oil were 1 and 2 mg/ml, respectively.

Conclusion: Role of meat as a reservoir for transmission of P. aeruginosa strains was determined in this survey. According to the high antimicrobial effects of *Z. multiflora* and its edible nature, its application as an edible film to extend the shelf-life of different meat-based products should consider in further researches.

Key words: Pseudomonas aeruginosa, virulence factors, oligotypes, raw meat, *Zataria multiflora*, antimicrobial effects.

Resumen

Antecedentes: *Pseudomonas aeruginosa* se considera un patógeno oportunista responsable de algunos casos de deterioro de los alimentos y de probables enfermedades de transmisión alimentaria. El presente estudio se realizó para evaluar la prevalencia, los caracteres de virulencia y la oligotípica de las cepas de *P. aeruginosa* aisladas de muestras de carne y evaluar los efectos antimicrobianos de *Zataria multiflora* contra los aislados.

Métodos: Se recogieron doscientas setenta muestras de carne cruda y se evaluó la presencia de *P. aeruginosa* mediante el cultivo. Los aislados fueron sometidos a secuenciación y oligotípica y también a un procedimiento de PCR para obtener los caracteres de virulencia. Las partes aéreas de *Z. multiflora* se utilizaron para preparar el aceite esencial. Se utilizó la cromatografía de gases para determinar los componentes químicos. Se utilizó la difusión en disco para evaluar los efectos antimicrobianos de *Z. multiflora*. También se evaluó la concentración inhibitoria mínima y la concentración bacteriana mínima de *Z. multiflora*.

Resultados: Doce de 270 (4,44%) muestras de carne estaban contaminadas con *P. aeruginosa*. La carne bovina cruda presentó la mayor prevalencia de *P. aeruginosa* (7,14%). ExoS (41,66%) fue el factor de virulencia más comúnmente detectado, mientras que algD fue el más raro (8,33%). Se detectaron cuatro oligotipos en los aislados de *P. aeruginosa*. El oligo_7 (33,33%) tuvo la mayor distribución. El timol (22,75%), el carvacrol (15,81%), el óxido de cariofileno (8,84%) y el a-pineno (7,73%) fueron los compuestos fitoquímicos más identificados en el aceite esencial de *Z. multiflora*. El diámetro de la zona de inhibición del crecimiento de los aislados de *P. aeruginosa* tratados con el aceite esencial de *Z. multiflora* (1%) fue estadísticamente superior al de la penicilina, la gentamicina, la ampicilina y la tetraciclina ($P< 0,05$) e insignificantemente inferior al de la azitromicina ($P> 0,05$). La CIM y la CBM de los aislados de *P. aeruginosa* tratados con aceite esencial de *Z. multiflora* fueron de 1 y 2 mg/ml, respectivamente.

Conclusiones: En este estudio se determinó el papel de la carne como reservorio para la transmisión de cepas de *P. aeruginosa*. Debido a los elevados efectos antimicrobianos de *Z. multiflora* y a su naturaleza comestible, su aplicación como película comestible para prolongar la vida útil de diferentes productos cárnicos debería considerarse en futuras investigaciones.

Palabras clave: Pseudomonas aeruginosa, factores de virulencia, oligotipos, carne cruda, *Zataria multiflora*, efectos antimicrobianos.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an unscrupulous bacterium related to both humans and animals. The bacterium is a causative agents in different infectious diseases, such as respiratory tract, urinary tract, gastric tract, burn and wound, bone and joint, and soft tissue infections, and bacteremia in hospitalized patients[1].

The bacterium has some responsibilities in the procedure of food spoilage and contamination, especially in meat samples. This procedure caused several bad effects on flavor, odor, and color of meat and derived products. In keeping with this, there is no available data about the foodborne aspects of the *P. aeruginosa*. However, consumption of food contained the bacterium may be result in gastrointestinal disorders[2].

P. aeruginosa has so many virulence factors with important responsibilities in the pathogenesis of infections. Among them, exoenzymes (*exoS*), elastase gene (*las*), and alginate-encoded genes (*algD* and *algU*) are considered important factors in adhesion and invasion[3].

Taxonomic classification of *P. aeruginosa* strains has so many advantageous in epidemiological studies. Oligotyping, which decays a given taxon, or 97% operational taxonomic units (OUT), into high-resolution units ('oligotypes') by only using the most information-rich nucleotide positions identified by Shannon entropy calculations is one of the best techniques for taxonomic classification[4]. Application of this technique as an analytical tool for studies of microbial ecology have provided the possibility for in-depth researches of microbial diversity in food and food-related environments[5].

P. aeruginosa has an emergence of antibiotic resistance[6]. Thus, scientists attracted to the synthesis of novel antimicrobial agents based on plant materials and essential oils. *Zataria multiflora* (*Z. multiflora*) is a medicinal plant belonging to the Laminaceae family. It grows in Iran, Pakistan and Afghanistan and is known as Shirazi Avishan. This plant is known as potential edible antimicrobial agents. It application as an edible film for improve the shelf-life of different types of foods has been reported previously[7].

Rendering the high importance of *P. aeruginosa* the present survey was done to assess the virulence characters and oligotyping of *P. aeruginosa* strains isolated from meat and evaluation of the antimicrobial effects of *Z. multiflora* against isolates.

Materials and methods

Plant materials

Aerial parts of the *Z. multiflora* were collected from Isfahan province. Plants were confirmed by an expert professor of the field of medicinal plants. The aerial parts of plant were dried in shade at room temperature. They were then ground. The essential oil was obtained by hydro-distilling of ground material with boiling water up to 4 h utilizing a Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulfate followed by filtering and stored at 4ºC in sealed glass vials for further use.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC/MS analyses were done using Hewlett–Packard 6890N gas chromatograph equipped with a column HP-5MS (30 m length, 0.25 mm diameter., and 0.25 lm film thickness) coupled with a Hewlett–Packard 5973N mass spectrometer. The column temperature was programmed at 50ºC, holding for 6 min, with 3ºC increases per min to reach to a final the temperature of 240ºC, followed by a temperature enhancement of 15ºC per min up to 300ºC and holding for 3 min. Injector port temperature was 290ºC and helium used as carrier gas at a flow rate 1.5 ml/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250ºC. The individual compounds were identified and confirmed thereafter of Kovats or retention indices calculation of components relative to their retention times of a series of n-alkanes and comparing them and their mass spectra with those of authentic samples or with available library data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra[8].

Meat samples

A total of 270 raw bovine (n= 70), ovine (n= 70), caprine (n= 50), and camel (n= 80) meat samples were collected from Isfahan province (25 g each in a sterile plastic bag). Samples were transferred to laboratory in 4ºC. For unique conditions, the thigh muscle was used for sampling.

P. aeruginosa isolation and identification

Twenty-five grams of meat samples were put in sterile Stomacher bags contained 225 ml peptone water (Oxoid, Basingstoke, UK). The bags underwent maceration within the stomacher (Seward 400 circulator) for 4 min at 260 beats per min. A total of 100 µl homogenate samples were placed on CN Selective Agar (Oxoid SR 102E, UK) supplemented with Pseudomonas Agar base (Oxoid, UK). Media were incubated at 37ºC for 24 h in aerobic conditions. Pseudomonas were identified by microscopic morphology, catalase, oxidase and urease activity, casein and starch hydrolysis, citrate and indole utilization, and Methyl Red-Voges Proskauer and gelatin liquefaction tests, using standard microbial techniques. Additionally, API 20NE strips (BioMeriouxVitek, Inc., MO, USA) system was used to identify the *P. aeruginosa*.

DNA extraction and PCR examination of virulence factors

DNA was extracted from the bacterial colonies using the cinnamene DNA extraction kit (Cinnagen, Iran)

Table I: PCR used to detect virulence factors[17,18].

<table>
<tr><th>Targeted genes</th><th>Primer sequence (5'-3')</th><th>PCR product (bp)</th><th>PCR programs</th><th>PCR volume (50µL)</th></tr>
<tr><td>algD</td><td>F:AAGGCGGAAATGCCATCTCC
R: AGGGAAGTTCCGGGCGTTTG</td><td>275</td><td rowspan="2">1 cycle:
2 min: 95^{0C}
30 cycles:
30s: 94^{0C}
30s: 58^{0C}
60s: 72^{0C}
1 cycle:
7 min: 72^{0C}</td><td rowspan="2">10X PCR buffer: 5 µL
Mgcl$_2$: 1.5 mM
dNTP: 200 µM
Primer F: 0.5 µM
Primer R: 0.5 µM
Taq DNA polymerase: 1.25 U
DNA: 2.5 µL</td></tr>
<tr><td>algU</td><td>F: CGCGAACCGCACCATCGCTC
R: GCCGCACGTCACGAGC</td><td>410</td></tr>
<tr><td>lasB</td><td>F: ACACAATACATATCAACTTCGC
R: AGTGTGTTTAGAATGGTGATC</td><td>284</td><td rowspan="2">1 cycle:
3 min: 94^{0C}
30 cycles:
30 s: 94^{0C}
60 s: 55^{0C}
90 s: 72^{0C}
1 cycle:
5 min: 72^{0C}</td><td rowspan="2">10X PCR buffer: 5 µL
Mgcl$_2$: 1.5 mM
dNTP: 200 µM
Primer F: 0.5 µM
Primer R: 0.5 µM
Taq DNA polymerase: 1.25 U
DNA: 2.5 µL</td></tr>
<tr><td>exoS</td><td>F: GTGTGCTTTATGCCATGAG
R: GGTTTCCTTTTCCAGGTC</td><td></td></tr>
</table>

according to method described by instruction[9,10]. Quality and quantity of extracted DNA were then checked[11-13]. **Table I** shows the PCR conditions met to detect virulence factors. A thermocycler (Eppendorf, Germany) was used in all PCR reactions. Electrophoresis was done according to previous researches[14-16].

Oligotypes detection

At first sequencing of isolates was done according to method described by Stellato et al. (2017)[19]. For oligotyping, 308 and 842 quality-controlled V1-V3 reads from bacteria isolates were used. Raw reads were quality-filtered as follows: reads were trimmed at the first ambiguous base or when the average quality score dropped below 25 within a 50-bp-long window, and reads shorter than 500 bp and with >1 primer mismatch were discarded. The PyNAST algorithm aligned the high-quality 454 reads against the GreenGenes multiple sequence alignment template and alignment was further trimmed to equal length by using the o-smart-trim script included in the oligotyping package v. 1.0. Global Assignment of Sequence Taxonomy (GAST) algorithm was used to identify "Pseudomonas." Following the initial entropy analysis oligotyping was performed using version 2.1 of the oligotyping pipeline1 using a total of 14 positions with high entropy, chosen to compute the oligotypes (-C option). After removal of oligotypes that did not meet these criteria, the analysis retained 299,055 reads (88.765% of the original reads). Oligotyping analysis identified 15 Pseudomonas oligotypes, representative sequences of which had at least one perfect match (100% sequence identity over 100% of query alignment) to rRNA gene entries in NCBI non-redundant (nr) database.

Antimicrobial effects of Z. multiflora

The simple disk diffusion method was used to assess the antimicrobial effects of *Z. multiflora* against *P. aeruginosa* isolates. For this purpose, isolated bacteria were cultured on Muller Hinton agar media. A total of 1000 µl of 1% *Z. multiflora* essential oil were poured into the blank disk and located at the surface of each media. For comparison, tetracycline (30 µg/disk), penicillin (10 µg/disk), gentamicin (10 µg/disk), azithromycin (15 µg/disk), and ampicillin (10 µg/disk) (Oxoid, UK) antibiotic disks were accompanies. All guidelines were performed according to the Clinical and laboratory standard institute (CLSI)[20-22]. The Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Z. multiflora* essential oil were also assessed. For this purpose, 0.5, 1, 2, and 4 mg/ml concentrations of *Z. multiflora* essential oil were prepared and the MIC and MBC values were determined using the previously described method[23].

Data analysis

Data analysis was performed by SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's exact two-tailed tests were performed to assess any significant relationship[24,25]. Besides, p-value < 0.05 was considered statistically significant[26,27].

Results

P. aeruginosa distribution

Table II shows the *P. aeruginosa* distribution amongst examined meat samples. Total contamination rate of meat samples with the *P. aeruginosa* was 4.44% (12/270). Raw bovine meat harbored the highest prevalence of contamination with *P. aeruginosa* (7.14%), while raw camel meat harbored the lowest (1.25%). Statistically significant differences were observed between type of samples and *P. aeruginosa* distribution (P< 0.05).

Table II: *P. aeruginosa* distribution amongst examined meat samples.

Meat samples	N. collected	N. positive for P. aeruginosa (%)
Bovine	70	5 (7.14)
Ovine	70	3 (4.28)
Caprine	50	3 (6)
Camel	80	1 (1.25)
Total	270	12 (4.44)

P. aeruginosa virulence characters

Figure 1 shows the PCR electrophoresis of the virulence factors of *P. aeruginosa* isolates.

Figure 1: PCR gel electrophoresis of the virulence factors.

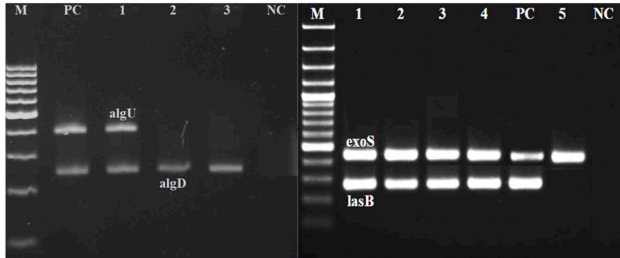


Table III shows the virulence factors distribution amongst the *P. aeruginosa* isolates of meat samples. *ExoS* (41.66%) was the most commonly detected virulence factors, while *algD* was the rarest (8.33%). *P. aeruginosa* isolates of raw bovine meat harbored the highest and most diverse profile of virulence factors. Statistically significant differences were observed between type of samples and *P. aeruginosa* virulence factors distribution ($P < 0.05$).

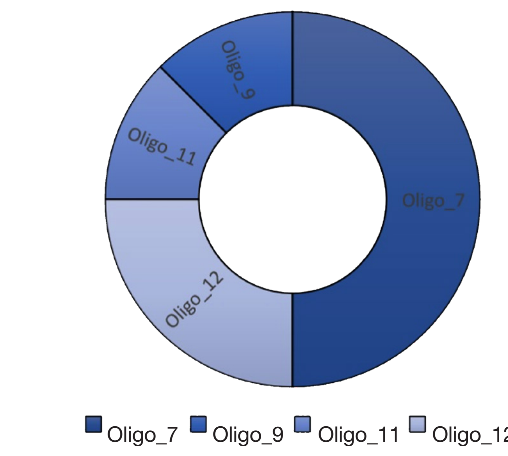
Table III: Virulence factors distribution amongst the *P. aeruginosa* isolates.

Meat	N. positive	N. isolates harbored each virulence factors samples (%)			
		algD	*algU*	*lasB*	*exoS*
Bovine	5	2 (40)	1 (20)	2 (40)	2 (40)
Ovine	3	1 (33.33)	-	1 (33.33)	1 (33.33)
Caprine	3	-	-	1 (33.33)	1 (33.33)
Camel	1	-	-	-	1 (100)
Total	12	3 (25)	1 (8.33)	4 (33.33)	5 (41.66)

P. aeruginosa oligotypes

Figure 2 shows the oligotypes abundance amongst the *P. aeruginosa* strains isolated from different meat samples. Four different oligotypes were identified amongst the isolates. Our findings showed that the Oligo_7, Oligo_9, Oligo_11, and Oligo_12 types were detected in 33.33%, 8.33%, 8.33%, and 16.66% of isolates.

Figure 2: Oligotypes abundance amongst the *P. aeruginosa* strains isolated from different meat samples.



Z. multiflora phytochemical analysis

Table IV shows the GC-MS analysis of the phytochemical compounds of the *Z. multiflora* essential oil. A total of 13 phytochemical compounds were detected in *Z. multiflora* essential oil (93.81%). Thymol (22.75%), carvacrol (15.81%), caryophyllene oxide (8.84%), a-Pinene (7.73%), a-Terpineol (7.19%), and linalool (7.09%) were the most commonly identified phytochemical compounds in the *Z. multiflora* essential oil.

Table IV: GC-MS analysis of the phytochemical compounds of the *Z. multiflora* essential oil.

No	Chemical compounds	Frequency (%)
1	p-Cymene	4.39
2	Cis-Sabinene hydrate	1.12
3	Borneol	2.71
4	Thymol	22.75
5	Carvacrol	15.81
6	a-Pinene	7.73
7	b-Myrcene	1.92
8	a-Phellandrene	5.37
9	a-Terpineol	7.19
10	Eugenol	2.28
11	Spathlenol	6.61
12	Linalool	7.09
13	Caryophyllene oxide	8.84
	Total	93.81

Antimicrobial effects of *Z. multiflora*

Table V shows the growth inhibition zone of *P. aeruginosa* isolates. The diameter of the growth inhibition zones had the ranges between 14.55±1.17 to 5.92±0.40 mm. The dimeter of the growth inhibition zone of *P. aeruginosa* isolates treated with *Z. multiflora* (1%) essential oil was 13.00±1.08 mm. The dimeter of the growth inhibition zone of *P. aeruginosa* isolates treated with *Z. multiflora* (1%) essential oil was statistically higher than penicillin, gentamicin, ampicillin, and tetracycline ($P < 0.05$) and insignificantly lower than azithromycin ($P > 0.05$).

Table V: Growth inhibition zone of *P. aeruginosa* isolates.

Tested antimicrobial agents	Diameter of the growth inhibition zone of *P. aeruginosa* isolates (mm)
Z. multiflora (1%)	$13.00±1.08^{a*}$
Tetracycline	$6.28±0.41^{c}$
Penicillin	$6.88±0.27^{c}$
Gentamicin	$5.92±0.40^{c}$
Azithromycin	$14.55±1.17^{a}$
Ampicillin	$8.33±0.71^{b}$

Table VI shows the MIC and MBC of *P. aeruginosa* isolates treated with *Z. multiflora* (1%) essential oil. The MIC and MBC of *P. aeruginosa* isolates treated with *Z. multiflora* essential oil were 1 and 2 mg/ml, respectively.

Table VI: MIC values of *Z. multiflora* against *P. aeruginosa* isolates.

Treatment	MIC (mg/ml)	MBC (mg/ml)
Z. multiflora	1	2

Discussion

Scarce data are available about the isolation of *P. aeruginosa* from meat samples. In this survey, total prevalence of *P. aeruginosa* amongst the examined raw bovine, ovine, caprine and camel meat samples was 7.14%, 4.28%, 6% and 1.25%. from surveys conducted in this field previously, majority of them assess the *P. aeruginosa* distribution amongst fish and meat products[28,29]. Previous survey in west Africa[30] showed that the prevalence of *P. aeruginosa* amongst the bovine meat samples was 53.04%. in previous researches presence of *P. aeruginosa* strains was reported from chicken meat (46.70%)[31], camel meat (80.00%)[32], and retail meat (3.00%)[33]. Differences in prevalence maybe due to differences in the hygienic conditions of slaughterhouses in different countries.

P. aeruginosa isolates harbored several virulence factors, including *exoS, lasB, algD*, and *algU*. It may show their virulent nature. These genes are mainly responsible for the adhesion and invasion of bacteria into the host cells. In keeping with this, consuming meat containing virulent *P. aeruginosa* strains may cause severe food-borne infection. However, there were no previously published data about the distribution of *P. aeruginosa* virulence factors amongst food samples. Oligotyping of strains (Oligo_7, Oligo_9, Oligo_11, and Oligo_12) showed similar taxonomy of our isolates with those of Pseudomonas sp. isolated from meat, dairy samples and environment[19]. This finding may show their similar source of contamination of isolated *P. aeruginosa* strains.

Z. multiflora medicinal plant had the boost antimicrobial effects against *P. aeruginosa* strains. This matter maybe partly due to the antimicrobial compounds found in the pants, such as thymol (22.75%), carvacrol (15.81%), caryophyllene oxide (8.84%), a-Pinene (7.73%), a-Terpineol (7.19%), and linalool (7.09%). All of these phytochemical components were found in other surveys as antimicrobial agents of the *Z. multiflora* essential oil[34,35]. As showed the *Z. multiflora* had higher antimicrobial effects against *P. aeruginosa* strains than other antimicrobial agents (except vancomycin). In the authors opinions, its antimicrobial effects should be higher that those reported in this study. It is because of the hard and strict lipopolysaccharide wall of the *P. aeruginosa* strains as a Gram-negative bacteria.

Conclusion

Findings of this paper showed that raw meat may consider as a reservoir of virulent and resistant *P. aeruginosa*. Additionally, isolates were mainly susceptible toward *Z. multiflora* essential oil. This matter may pose the application of *Z. multiflora* essential oil as an edible antimicrobial compound to extend the shelf life of foods, especially meat samples and derived products in the food industry. Role of food as a vector for transmission of *P. aeruginosa* should consider in further studies.

Conflict of interest

Authors do not have any conflict of interest to declare.

References

1. Spagnolo AM, Sartini M, Cristina ML. Pseudomonas aeruginosa in the healthcare facility setting. Reviews in Medical Microbiology. 2021;32(3):169-75.

2. Kolbeck S, Abele M, Hilgarth M, Vogel RF. Comparative Proteomics Reveals the Anaerobic Lifestyle of Meat-Spoiling Pseudomonas Species. Frontiers in Microbiology. 2021;12:695.

3. Jurado-Martín I, Sainz-Mejías M, McClean S. Pseudomonas aeruginosa: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. International Journal of Molecular Sciences. 2021.

4. De Filippis F, Pellegrini N, Laghi L, Gobbetti M, Ercolini D. Unusual sub-genus associations of faecal Prevotella and Bacteroides with specific dietary patterns. Microbiome. 2016 Dec;4(1):1-6.

5. Schmidt VT, Reveillaud J, Zettler E, Mincer TJ, Murphy L, Amaral-Zettler LA. Oligotyping reveals community level habitat selection within the genus Vibrio. Frontiers in microbiology. 2014 Nov 13;5:563.

6. Algammal AM, Mabrok M, Sivaramasamy E, Youssef FM, Atwa MH, El-Kholy AW, et al. Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes. Scientific reports. 2020 Sep 29;10(1):1-2.

7. Hashemi SA, Azadeh S, Nouri BM, Navai RA. Review of pharmacological effects of Zataria multiflora Boiss.(thyme of Shiraz). International Journal of Medical Research & Health Sciences. 2017;6(8):78-84.

8. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured publishing corporation; 2007.

9. Dehkordi FS, Saberian S, Momtaz H. Detection and segregation of Brucella abortus and Brucella melitensis in aborted bovine, ovine, caprine, buffaloes and camelid fetuses by application of conventional and real-time polymerase chain reaction. The Thai Journal of Veterinary Medicine. 2012;42(1):13.

10. Dehkordi FS, Momtaz H, Doosti A. Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. Bulgarian Journal of Veterinary Medicine. 2012;15(1):30-6.

11. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011;6(4):180-6.

12. Dehkordi FS, Tavakoli-Far B, Jafariaskari S, Momtaz H, Esmaeilzadeh S, Ranjbar R, et al. Uropathogenic Escherichia coli in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infections. 2020;38:100824.

13. Dehkordi FS, Haghighi N, Momtaz H, Rafsanjani MS, Momeni M. Conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. Bulgarian Journal of Veterinary Medicine. 2013;16(2):102-12.

14. Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes. 2014c;7(1):1-8.

15. Dehkordi FS, Barati S, Momtaz H, Ahari SN, Dehkordi SN. Comparison of shedding, and antibiotic resistance properties of Listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species in Iran. Jundishapur Journal of Microbiology. 2013a;6(3):284.

16. Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017;6(1):1-1.

17. Finnan S, Morrissey JP, O'gara F, Boyd EF. Genome diversity of Pseudomonas aeruginosa isolates from cystic fibrosis patients and the hospital environment. Journal of Clinical Microbiology. 2004 Dec;42(12):5783-92.

18. Gorgani N, Ahlbrand S, Patterson A, Pourmand N. Detection of point mutations associated with antibiotic resistance in Pseudomonas aeruginosa. International Journal of Antimicrobial Agents. 2009 Nov 1;34(5):414-8.

19. Stellato G, Utter DR, Voorhis A, De Angelis M, Eren AM, Ercolini D. A few Pseudomonas oligotypes dominate in the meat and dairy processing environment. Frontiers in microbiology. 2017 Mar 2;8:264.

20. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 28th Informational Supplement. CLSI Document M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute. 2018.

21. Safarpordehkordi F, Yahaghi E, Khodaverdi Darian E. Prevalence of antibiotic resistance in Escherichia coli isolated from poultry meat supply in Isfahan. Iranian Journal of Medical Microbiology. 2014;8(2):41-7.

22. Ranjbar R, Seif A, Dehkordi FS. Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic Escherichia coli recovered from hospital food. Jundishapur J Microbiol. 2019;12(5):8.

23. Dadashi M, Hashemi A, Eslami G, Fallah F, Goudarzi H, Erfanimanesh S, et al. Evaluation of antibacterial effects of Zataria multiflora Boiss extracts against ESBL-producing Klebsiella pneumoniae strains. Avicenna journal of phytomedicine. 2016 May;6(3):336.

24. Dehkordi FS, Valizadeh Y, Birgani TA, Dehkordi KG. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. Journal of Pure and Applied Microbiology. 2014b;8(2):1065-9.

25. Dehkordi FS, Tirgir F, Valizadeh Y. Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. Veterinary Research Forum. 2017; 8(3):215.

26. Nejat S, Momtaz H, Yadegari M, Nejat S, Safarpour Dehkordi F, Khamesipour F. Seasonal, geographical, age and breed distributions of equine viral arteritis in Iran. Kafkas Univ Vet Fak Derg. 2015;21(1):111-6.

27. Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Dehkordi FS. Genotypic and phenotypic-based assessment of antibiotic resistance and profile of staphylococcal cassette chromosome mec in the methicillin-resistant Staphylococcus aureus recovered from raw milk. Infection Drug Res. 2020;13:273.

28. Wickramasinghe NN, Ravensdale J, Coorey R, Chandry SP, Dykes GA. The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. Comprehensive Reviews in Food Science and Food Safety. 2019;18(5):1622-35.

29. Sheir SH, Ibrahim HM, Hassan MA, Shawky NA. Incidance of Psychotropic bacteria in frozen chicken meat products with special reference to Pseudomonas species. Benha Veterinary Medical Journal. 2020;39(1):165-8.

30. Benie C, Nathalie G, Adjéhi D, Solange A, Fernique K, Desire KN, et al. Prevalence and antibiotic resistance of Pseudomonas aeruginosa isolated from bovine meat, fresh fish and smoked fish. Archives of Clinical Microbiology. 2017;8(3).

31. Benie CD, Dadie A, Guessennd NK, Kouame ND, Yobouet BA, Aka S. Prevalence and diversity of Pseudomonas sp. isolated from beef, fresh and smoked fish in Abidjan Cote de'voire. Journal of Food and Dairy Technology. 2016;4(4):2347-59.