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Short Communication: Molecular study of bacteria isolated from meat and chicken frozen from Misan Governorate market in Iraq

DUAA ALI HUSAIN, ZAHID SA'DOON AZIZ♥
Department of Biology, College of Science, University of Misan. Amarah, Iraq.♥email: dr.aziz_zoonobiology@uomisan.edu.iq

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Abstract. *Husain DA, Aziz ZS. 2021. Short Communication: Molecular study of bacteria isolated from meat and chicken frozen from Misan Governorate market in Iraq. Biodiversitas 23: 81-86.* Food contamination is frequently defined as spoiled or tainted foods because they contain microorganisms, such as fungi, bacteria, parasites, or toxic substances that make them unfit for consumption. In this study, one hundred meat and chicken Frozen products samples were collected randomly from the commercial markets in Misan Governorate, Iraq from November 2020 to April 2021, included: (burger – sausage – kebab – shawarma – minced meat) of meat and (chest-thigh-liver-burger-kebab) of Chicken. The results of aerobic plate count showed that all meat and chicken products were contaminated with bacteria, but the imported products were more contaminated than the local products. Our study included the isolation and diagnosis of eighteen bacterial species by using routine and standard bacteriological tests. The results showed that Gram-negative bacteria were common in meat products (52%) and Gram-positive bacteria represented 48%. In chicken samples, the Gram-positive bacteria was most dominant (59%) while the percentage of Gram-negative bacteria was 41%. Monoplex PCR was used to identify eighteen bacterial strains using a universal 16S rRNA primer that gave 1500 bp amplification product, nucleotide sequences were studied at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (Nucleotide BLAST). The diagnosed bacteria were *Aeromonas veronii, Pseudomonas plecoglossicida, Acinetobacter lwofii, Aeromonas veronii, Klebsiella pneumoniae, Pseudomonas japonica, Pseudomonas songnenensis, Klebsiella pneumoniae* subsp. *ozaenae, Psychrobacter sanguinis, Klebsiella pneumoniae* strain, *Acinetobacter lwofii, Lysinibacillus boronitolerans, Bacillus licheniformis, Enterobacter hormaechei, Pseudomonas putida* strain, *Serratia liquefaciens* strain, *Comamonas testosterone* strain, and *Methylogaea oryzae* strain which recorded in the Gene Bank belong to different accession numbers.

Keywords: 16SrRNA, food contamination, monoplex PCR, spoiled

INTRODUCTION

Public health is at risk from foodborne pathogens, leading to illness (Akeda 2015). There are an estimated 600 million foodborne illness cases each year, making them a major public health issue (Srey et al. 2013; WHO 2020). Bacteria, fungi, and parasites are some microorganisms that can infect humans through food or water known as foodborne pathogens (FPPs) (Dwivedi and Jayku 2011). In recent years, foodborne pathogens have become a major public health concern worldwide, resulting in a high morbidity and mortality rate (Oliver et al. 2005; Zhao et al. 2014). According to Sadiku et al. (2020), physical or foreign material, chemical, and biological contamination all occur during food processing.

Spoilage and pathogenic bacteria are the two most important types of bacteria. There are usually no health risks associated with spoilage bacteria, but their presence can cause food to lose its freshness and quality (Pennacchia et al. 2011). When it comes to food-borne illness, pathogenic bacteria include those like *Salmonella*, *Escherichia coli* and *Campylobacter jejuni* and *Staphylococcus aureus* and *Listeria monocytogenes* (Abbas and Alghanim 2016). It is possible to safely store foods like meat, meat products, and poultry for extended periods of time if the proper hygiene procedures are followed. This includes the cleanliness of the surfaces that come into contact with food while processed. To preserve food, microbial enzymes and the natural enzymes found in food must be prevented from damaging it (Ledward 2003 and Rajendran et al. 2019). This study aimed to determine the percentages of bacterial contamination in frozen food sold in the public markets of Misan Governorate using biochemical and molecular methods.

MATERIALS AND METHODS

Collection of samples

One hundred samples of frozen food were collected randomly from the markets of Misan governorate, Iraq through six months from November 2020 to April 2021 included: meat products, represented by (burger – sausage – kebab – shawarma-minced meat) and chicken products, represented by (chest-thigh-liver-burger-kebab) where 5 samples for each product were used, the samples included local and imported companies**.**

Preparation of samples

As described by Khalafallah et al. (2020) with some modification under complete aseptic conditions, 25 g of the sample were weighed, cut into small pieces and then transferred into a sterile flask containing 225 mL of sterile peptone water (0.1%). The content of the flask was homogenized for three minutes at 14000 rpm. One mL from the homogenate was transferred into a separate tube containing nine mL of sterile peptone water (0.1%) from which tenfold serial dilutions were prepared. The prepared samples were subjected to the following examinations.

Aerobic plate count

After completing the serial dilutions, 0.1 mL of the dilution 10^{-10} was taken and deployed by a swab on the surface of the nutrient agar. The plates were inverted and incubated at 37ºC for 24 hours. After 24 hours bacterial colonies were counted by colony counter. The CFU/mL was calculated as below :

CFU per mL: No. of colonies *Inverted dilution factor/ inoculum volume (Hafez et al. 2020).

Identification of bacteria

Preliminary tests

The morphological characteristics of the growing colonies of bacteria were determined to include color, size, form, elevation and margin of the colonies on ordinary, enrichment, selective and differential media (Nutrient agar, Blood agar and MacConkey agar) (Goldman and Lorrence 2009). Gram stain were used to differentiate shapes of bacterial isolates and to distinguish between Gram positive-negative bacteria.

Molecular identification

Molecular detection of 16S rRNA gene of bacteria isolated from frozen food was performed by Polymerase Chain Reaction (PCR) assay as in the following steps:

DNA extraction

Genomic DNA Mini Bacteria Kit (Presto™ Mini gDNA Bacteria Kit) was used to extract genomic DNA from bacteria according to company's instructions (Geneaid, Taiwan). The 16S rRNA primers prepared by Bioneer (South Korea) were used in this study as previously mentioned by Miyoshi et al. (2005): 27 F 5´-AGAGTTTGATCCTGGCTCAG 3´ and 1492 R 5´-GGTTACCTTGTTACGACTT 3´.

PCR amplification

As described by Miyoshi et al. (2005) with some modification all components of PCR were assembled in PCR tube and mixed by cooling microcentrifuge for 10 sec at 850 rpm. A PCR reaction mix was prepared using the AccuPower® PCR Master Mix kit, manufactured by the Bioneer Korean company. All PCR reactions were performed in a final volume of 25 µL using four µL of extracted DNA as template, 5 µL of master mix, forward primer one µL, reverse primer one µL, and molecular grade water 14 µL. Then PCR amplification was carried out in a thermocycler (Prime, UK) with the following thermal conditions: an initial denaturation at 94°C for one min, followed by 30 cycles each of one min denaturation at 94°C, 35 s annealing at 52°C, one min extension at 72°C, and final extension at 72°C for seven min, and finally the PCR product was held at four°C. The amplified PCR products were separated by electrophoresis (Consort, Belgium) in 1.5% agarose gel stained with ethidium bromide and visualized with a UV transilluminator (Electrofor, Italy).

Statistical analysis

Extract the mean and standard error of total aerobic plate count. The data were analyzed using the statistical program Social Package of Social Sciences (SPSS) version 22, using independent samples T-test to calculate the statistical differences (Al-Rawi and Khalf Allah 2000).

RESULTS AND DISCUSSION

Isolation and enumeration of bacteria by using total Aerobic Plate Count

Eighteen bacterial isolates were isolated from frozen food samples (meat and poultry product), numerated by using serial dilutions on the nutrient agar. It is easy to perform, and many organisms can be counted as CFU/mL (Sultana et al. 2014). Statistical analytical results of total aerobic plate count showed in Tables 1 and 2.

The results in Table 1 showed that the imported samples of three types of meat products had highest value of APC than local samples and there were significant differences ($P<0.05$) between imported and local burger and sausage samples while there were no significant differences ($P>0.05$) between imported and local shawarma samples.

The minced meat samples from local (L1) have mean value less than the samples of local (L2) so the samples from L2 had high values of APC than the L1 samples while kebab from L1 has to mean value more than the samples from L2 so the kebab samples from L1 have APC than the samples from L2 highly. There was significant difference ($P<0.05$) between L1 and L2 of kebab samples while there was no significant difference ($P>0.05$) between L1 and L2 of minced meat samples.

The Total Aerobic Plate Count (APC) reflects the bacterial contamination and declares the hygienic quality of meat and chicken products (Younes et al. 2019). As shown in Table 1, this investigation found bacteria in every type of meat sampled, but imported meats were more contaminated than local meats. This may be due to the lengthy storage period, exposure to thawing and freezing during preservation or the spread of bacteria in meat, finally contamination of used tools, and lack of attention to personal hygiene of workers (Abd El Tawab et al. 2015).

Hassanein et al. (2020) in their study compared imported and local frozen meat were their findings reveal that all of the samples examined, whether they were chilled or frozen, are unfit for human consumption because of poor hygiene. Samples of beef burger, luncheon, pastirma, and sausage were examined by Abbas et al. (2014), who discovered that each sample was contaminated with a

different type of microorganism.

The results in Table 2 showed that the imported liver, breast, thighs and burger had highest value of APC than that of local samples. The statistical analytical results revealed significant differences (P<0.05) between imported and local samples for each type of them. The APC of kebab samples from L2 is more than the APC of L1 samples and there was no significant difference (P>0.05) between kebab samples from L1and L2. We found that imported samples were significantly more contaminated than local ones (P<0.05) in the current study's findings (table 2), which showed that all samples of meat and poultry cuts and products were contaminated. Slaughtering, cutting, and freezing of poultry exposed to various physical factors such as heat and moisture may have caused this result, as could a failure by manufacturing workers to adhere to health and hygiene regulations.

A study by Al-Tamimi and AL-Khafaji (2021) compared the quality of imported frozen poultry meat with the quality of fresh local poultry meat. Frozen meat, neck, thigh and breast had the highest viable bacterial counts compared to fresh meat, breast, thigh and neck. Their findings showed some discrepancies, but both studies agreed that imported frozen poultry meat was more contaminated than local meat. Many researchers have studied (Bohaychuk et al. 2006; Haleem et al. 2013; Yar et al. 2020) the microorganisms (spoilage and pathogenic bacteria) that can be found in both imported and domestic poultry products.

Preliminary identification of bacteria

Several subcultures were done to obtain pure culture for identification bacteria. All bacterial isolates have shown good growth on the Blood agar with different patterns of hemolysis (Figure 1). The Gram-positive bacteria didn't grow on the MacConkey agar while the Gram-negative bacteria grew very well.

Gram stain was showed that Gram-negative bacteria were most common bacterial strains in meat products (52%) while Gram-positive bacteria represent (48%). In poultry samples, Gram stain showed that Gram-positive bacteria were most bacterial strain (59%) with (41%) the percentage of Gram-negative bacteria. The forms of bacteria were ranged from cocci, bacilli and variable pleomorphic.

Detection of bacteria by molecular techniques

The bacterial universal primer pair specific to 16SrRNA gene fragment was used to identify bacterial isolates; positive results were recorded for all bacterial selected with an amplification band corresponding to 1500 bp, confirming that all isolates were bacterial species (Figure 2). The result is represented in Table 3 which shows identity percentages ranging from 80-97% to 100%.

Table 1. Statistical analytical results of total Aerobic Plate Count (APC) in the examined samples of meat products (n=50)

Meat products	Type	Min*	Max*	Mean ± SD*
Burger	I	1.72×10^{13}	3.20×10^{13}	2.52×10^{13b} $\pm1.44\times10^{13}$
	L	8.30×10^{12}	2.13×10^{13}	2.32×10^{13} ᵇ $\pm1.06\times10^{13}$
Sausage	I	5.60×10^{12}	1.92×10^{13}	3.86×10^{13} ᵇ $\pm2.92\times10^{13}$
	L	2.57×10^{13}	2.98×10^{13}	2.85×10^{13} ᵇ $\pm0.16\times10^{13}$
Minced meat	L1	1.12×10^{13}	8.3×10^{12}	5.56×10^{12a} $\pm3.10\times10^{12}$
	L2	2.32×10^{13}	3.3×10^{13}	2.59×10^{13} ᵃ$\pm0.4\times10^{13}$
Shawarma	I	1.28×10^{13}	4.1×10^{12}	2.78×10^{13} ᵃ$\pm1.12\times10^{13}$
	L	1.25×10^{13}	6.6×10^{12}	2.99×10^{13} ᵃ$\pm2.18\times10^{13}$
Kebab	L1	1.87×10^{13}	2.91×10^{13}	2.46×10^{13} ᵇ $\pm1.81\times10^{13}$
	L2	3.10×10^{12}	2.67×10^{13}	1.24×10^{13} ᵇ $\pm 0.19\times10^{13}$

Note: SD*: standard deviation of mean, Min*: minimum, Max*: maximum, I: imported, L: local. The value represents (mean± SD), Vertically similar letters indicate that there are no significant differences (P>0.05), different letters vertically between the values indicate that there are significant differences (P<0.05)

Table 2. Statistical analytical results of total Aerobic Plate Count (APC) in the examined samples of poultry cuts and products (n=50)

Poultry cuts and products	Type	Min.	Max.	Mean ± SD*
Liver	I	6.90×10^{12}	2.06×10^{13}	1.89×10^{13} ᵇ $\pm1.22\times10^{13}$
	L	8.80×10^{12}	9.50×10^{12}	9.2×10^{12} ᵇ $\pm4.7\times10^{12}$
Chest	I	2.45×10^{13}	3.73×10^{13}	2.91×10^{13} ᵇ $\pm2.16\times10^{13}$
	L	7.80×10^{12}	2.56×10^{13}	1.83×10^{13} ᵇ $\pm1.19\times10^{13}$
Thighs	I	2.54×10^{13}	3.00×10^{13}	2.78×10^{13} ᵇ $\pm1.26\times10^{13}$
	L	7.30×10^{12}	2.93×10^{13}	1.64×10^{13} ᵇ $\pm0.13\times10^{13}$
Kebab	L1	8.50×10^{12}	2.99×10^{13}	2.09×10^{13} ᵃ $\pm1.23\times10^{13}$
	L2	1.97×10^{13}	2.81×10^{13}	2.23×10^{13} ᵃ $\pm1.49\times10^{13}$
Burger	I	1.98×10^{13}	3.00×10^{13}	2.59×10^{13} ᵇ $\pm2.07\times10^{13}$
	L	1.55×10^{13}	2.01×10^{13}	1.66×10^{13} ᵇ $\pm1.29\times10^{13}$

Note: SD*: standard deviation of mean, Min*: minimum, Max*: maximum, I: imported, L: local. The value represents (mean± SD), Vertically similar letters indicate that there are no significant differences (P>0.05), different letters vertically between the values indicate that there are significant differences (P<0.05)

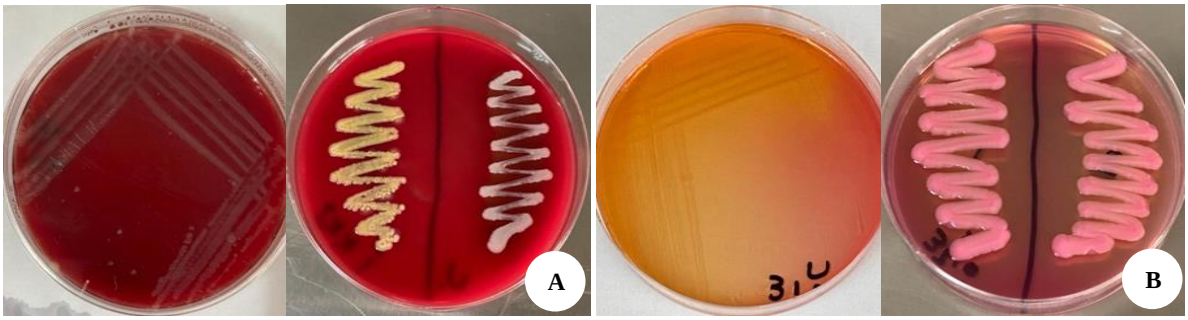

Figure 1. A. The bacterial growth on the Blood agar. B. The bacterial growth on the MacConkey agar

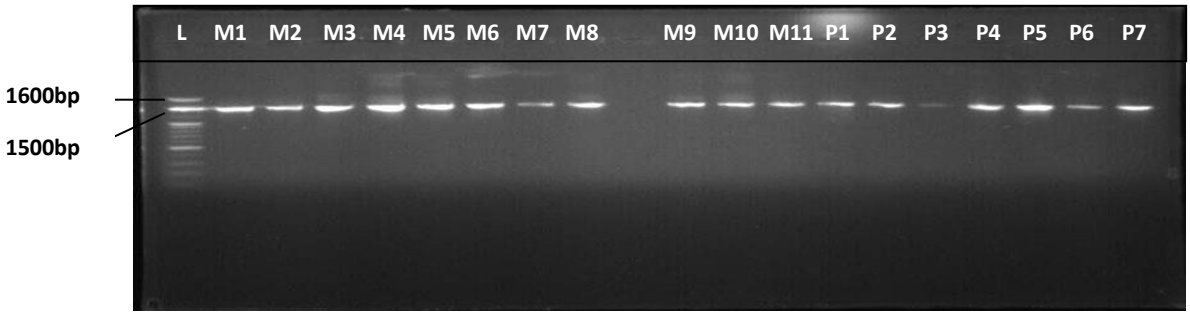

Figure 2. Ethidium bromide-stained gel electrophoresis of the 16s rRNA gene of bacterial strains (1500) lane (L) represents the molecular ladder (100bp) and lanes (M1-M11) (P1-P7) represents positive PCR results

Table 3. Bacterial identification based on 16S rRNA sequencing data

Code	Bacterial strains	Identity percent age	Accession no.
M1	*Pseudomonas songnenensis*	95.94%	NR-148295.1
M2	*Pseudomonas japonica*	99.71%	NR-114192.1
M3	*Klebsiella pneumoniae* subsp. *ozaenae*	98.82%	NR-041750.1
M4	*Klebsiella pneumoniae*	99.42%	NR-117683.1
M5	*Pseudomonas putida* strain	96.54%	NR-114794.1
M6	*Serratia liquefaciens* strain	95.82%	NR-121703.1
M7	*Klebsiella pneumoniae* strain	95.62%	NR-113702.1
M8	*Bacillus licheniformis* strain	99.66%	NR-165685.1
M9	*Pseudomonas plecoglossicida* strain	97.98%	NR-114226.1
M10	*Lysinibacillus boronitolerans* strain	94.14%	NR-114207.1
M11	*Enterobacter hormaechei* sub sp. *xiangfangensis*	96.25%	NR-126208.1
P1	*Psychrobacter sanguinis*	95.3%	NR-117833.1
P2	*Aeromonas veronii be.veronii* strain	97%	NR-119045.1
P3	*Aeromonas veronii be.veronii*	83.65%	NR-119045.1
P4	*Acinetobacter lwoffii* strain	97.79%	NR-026209.1
P5	*Acinetobacter lwoffii* strain	98.44%	NR-113346.1
P6	*Comamonas testosteroni* strain	100%	NR-029161.2
P7	*Methylogaea oryzae* strain	80.67%	NR-116407.1

The current study was able to record sixteen bacterial strains in Gene Bank data belong different accession numbers, the recorded bacterial were *Aeromonas veronii*, *Pseudomonas plecoglossicida*, *Acinetobacter lwofii*, *Aeromonas veronii*, *Klebsiella pneumoniae*, *Pseudomonas japonica*, *Pseudomonas songnenensis*, *K. pneumoniae subsp. ozaenae*, *Psychrobacter sanguinis*, *K. pneumoniae* strain, *Acinetobacter lwofii*, *Lysinibacillus boronitolerans*, *Bacillus licheniformis*, *Enterobacter hormaechei*, *Pseudomonas putida* strain and *Serratia liquefaciens* strain and their accession numbers were (MZ934693.1, MZ934671.1, MZ931306.1, MZ931286.1, MZ930472.1, MZ927456.1, MZ927229.1, MZ923509.1, MZ923503.1, MZ921931.1, MZ920155.1, MZ919358.1, MZ919316.1, MZ919316.1, MZ913024.1, MZ911849.1) respectively.

The present study results in Table 3 showed the identification of bacteria by16S rRNA amplification using PCR technique. For the confirmation of bacterial species isolated from frozen meat and poultry products samples, many studies have used universal primers (16S rRNA) like (Gwida et al. 2014; Delpiazzo et al. 2021).

Sirghani et al. (2018) evaluated the incidence of *Yersinia enterocolitica* in chicken meat by using culture method on selective media and confirmation by PCR assay using 16S rRNA gene, they found that due to high accuracy and speed of PCR assay, it is a good alternative method for microbiological techniques.

Polymerase Chain Reaction was used by Hameed et al. (2021) to confirm the presence of *Escherichia coli*, *Staphylococcus*, and *Salmonella* in raw meat and meat products samples with varying percentages of 16S rRNA genes.

Yersinia enterocolitica, *Salmonella species*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, and *Clostridium botulinum* were among

the pathogens isolated by Madoroba et al. (2021) from meats and meat products, and PCR was used to confirm the presence of selected bacteria and results provide evidence of diverse and highly variable microbial communities in products of animal origin, which is important for food safety, food labeling, biosecurity, and shelf life-limiting spoilage by microorganisms.

According to the findings of this study, a number of previous studies have identified some bacteria that have been linked to foodborne illness or food spoilage, such as *B. licheniformis* (Mikkola et al. 2000), *K. pneumoniae* (Shon et al. 2013), *Aeromonas veronini* (Stratev et al. 2015), and *Pseudomonas spp.* (Ibrahim et al. 2016). (Gustavsson et al. 2011).

In conclusion, the present study displayed that frozen foods (meat products and chicken cuts and their products) contain many bacteria, although local products were contaminated lower than imported ones. This contamination might occur inside slaughterhouses and during the additional processing of meat, as well as the tools used and workers. Therefore, it is important to improve the health quality of meat (Ensure that it is fit for human consumption), whether it is imported or local, isolation and identification of many pathogens and spoilage bacteria which cause great public health concern due to its zoonotic transmission to humans and cause spoilage of food by changing its taste and smell, that caused the economic losses in many countries, the results showed the properties of Monoplex PCR method which it is better than traditional methods. Moreover, traditional methods should be replaced by advanced molecular methods, because they are reliable, fast, sensitive, and labor-saving. The results recorded the pathogens and spoilage bacterial strains that might occur for the first time reported in Gene Bank in Iraq.

REFERENCES

Abbas AA, Samar Y ES. 2014. Microbiological criteria of some meat products. Alexandria J Vet Sci 42 (1): 83-89. DOI: 10.5455/ajvs.162116.

Abbas BA, Alghanim AM. 2016. Detection of virulence genes in Escherichia coli isolated from frozen meat in Basrah market. Basrah J Vet Res 15 (3): 134-138.

Abd El Tawab AA, El-Hofy FI, Maarouf AA, El-Said AA. 2015. Bacteriological studies on some food-borne bacteria isolated from chicken meat and meat products in Kaliobia Governorate. Benha Vet Med J 29 (2): 47-59. DOI: 10.21608/BVMJ.2015.31545.

Akeda Y. 2015. Food safety and infectious diseases. J Nutr Sci Vitaminol 61 (Supplement): S95-S95. DOI: 10.3177/jnsv.61.S95.

AL-Rawi KM, Khalf Allah AM. 2000. Design and Analysis of Agricultural Experiments. University of Mosul. Ministry of Higher Education and Scientific Research. Dar Al Kuttab for printing and publishing, Mosul, Iraq.

AL-Tamimi AH, AL-Khafaji NJ. 2021. Comparative study between imported frozen and local fresh poultry's meat in respect of Bacterial contamination with Enterobacteriaceae in Diyala province. Diyala J Vet Sci 1 (1): 1-18.

Bohaychuk VM, Gensler GE, King RK, Manninen KI, Sorensen O, Wu JT, McMullen LM. 2006. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. J Food Prot 69 (9): 2176-2182. DOI: 10.4315/0362-028X-69.9.2176.

Delpiazzo R, Barcellos M, Barros S, Betancor L, Fraga M, Gil J, Calleros L. 2021. Accurate and fast identification of Campylobacter fetus in bulls by real-time PCR targeting a 16S rRNA gene sequence. Vet Anim Sci 11: 100163. DOI: 10.1016/j.vas.2020.100163.

Dwivedi HP, Jaykus LA. 2011. Detection of pathogens in foods: the current state-of-the-art and future directions. Crit Rev Microbiol 37 (1): 40-63. DOI: 10.3109/1040841X.2010.506430.

Goldman E, Lorrence HG. 2009. Practical Handbook of Microbiology. (2nd Ed). CRC Press. DOI: 10.1201/9781420009330.

Gwida M, Hotzel H, Geue L, Tomaso H. 2014. Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. Intl Sch Res Notices 2014: 565671. DOI: 10.1155/2014/565671.

Gustavsson J, Cederberg C, Sonesson U, Otterdijk RV, Meybeck A. 2011. Global food losses and food waste. Extent, causes and prevention. Food and Agriculture Organization of the United Nations, Rome.

Hafez M, Ibrahim H, Amin R. 2020. Assessment of bacterial evaluation of imported frozen meat. Benha Vet Med J 37 (2): 1-3. DOI: 10.21608/BVMJ.2019.18547.1123.

Haleem AM, Al-bakri SA, Al-Hiyaly SA. 2013. Determination of microbial content in poultry meat in local Iraqi markets. Afr J Microbiol Res 3 (6): 205-207. DOI: 10.5923/j.microbiology.20130306.02.

Hameed MAK, Muhammed HA, Al-Rrashedi AAM. 2021. The bacterial load and contamination rate of raw meat in butcher shops in different areas of Karbala City. Pak J Med Health Sci 15 (March): 960. DOI: 10.1016/S014.

Hassanien F, Shaltout FAE, Fahmey MZ, Elsukkary HFA. 2020. Bacteriological quality guides in local and imported beef and their relation to public health. Benha Vet Med J 39 (1): 125-129. DOI: 10.21608/bvmj.2020.32598.1214.

Ibrahim SM, Abdelgadir MA, Sulieman AM. 2014. Impact of halal and non-halal slaughtering on the microbiological characteristics of broiler chicken meat and sausages. Food Publ Health 4 (5): 223-228. DOI: 10.5923/j.fph.20140405.03.

Khalafallah BM, El-Tawab A, Awad A, Nada S, Elkhayat ME. 2020. Phenotypic and genotypic characterization of pseudomonas species isolated from frozen meat. Benha Vet Med J 39 (2): 47-51. DOI: 10.21608/bvmj.2020.46777.1285.

Ledward DA. 2003. "Meat Preservation". Encyclopedia of Food Sciences and Nutrition. Academic Press, London. DOI: 10.1016/B0-12-227055-X/00752-5.

Madoroba E, Magwedere K, Chaora NS, Matle I, Muchadeyi F, Mathole MA, Pierneef R. 2021. Microbial communities of meat and meat products: an exploratory analysis of the product quality and safety at selected enterprises in South Africa. Microorganisms 9 (3): 507. DOI: 10.3390/microorganisms9030507.

Mikkola R, Kolari M, Andersson MA, Helin J, Salkinoja-Salonen MS. 2000. Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*. Eur J Biochem 267 (13): 4068-4074. DOI: 0.1046/j.1432-1033.2000.01467.x.

Miyoshi T, Iwatsuki T, Naganuma T. 2005. Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2 micrometer-pore-size filters. Appl Environ Microbiol 71 (2): 1084-1088. DOI: 10.1128/AEM.71.2.1084-1088.2005.

OliverSP, Jayarao BM, Almeida RA. 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2 (2): 115-129. DOI: 10.1089/fpd.2005.2.115.

Pennacchia C, Ercolini D, Villani F. 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiol 28 (1): 84-93. DOI: 10.1016/j.fm.2010.08.010.

Rajendran C, Mahesh S, Jayathilakan K. 2019. Advances in meat preservation and safety. Intl J Sci Res 10: 1499-1502. DOI: 10.21275/SR20326194523.

Sadiku MN, Ashaolu TJ, Musa SM. 2020. Food contamination: a primer. Intl J Adv Sci Res Eng 6 (3): 1-7. DOI: 10.31695/IJASRE.2020.33736.

Sirghani K, Zeinali T, Jamshidi A. 2018. Detection of *Yersinia enterocolitica* in retail chicken meat, Mashhad, Iran. J Pathog 2018: 1286216. DOI: 10.1155/2018/1286216.

Shon AS, Bajwa RP, Russo TA. 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence 4 (2): 107-118. DOI: 10.4161/viru.22718.

Srey S, Jahid IK, Ha SD. 2013. Biofilm formation in food industries: a food safety concern. Food Control 31 (2): 572-585. DOI: 10.1016/j.foodcont.2012.12.001.

Stratev D, Vashin I, Daskalov H. 2015. Microbiological status of fish products on retail markets in the Republic of Bulgaria. Intl Food Res J 22 (1): 64-69.

Sultana F, Afroz H, Jahan A, Fakruddin M, Datta S. 2014. Multi–antibiotic resistant bacteria in frozen food (ready to cook food) of animal origin sold in Dhaka, Bangladesh. Asian Pac J Trop Biomed 4 (1): S268-S271. DOI: 10.12980/APJTB.4.2014B85.

World Health Organization. 2020. Clinical consortium on healthy aging 2019: report of consortium meeting held 21-22 November 2019, Geneva, Switzerland.

Yar DD, Kwenin WK, Zanu WK, Balali GI, Adepa EK, Gyapong F. 2020. Microbial quality of frozen chicken parts from three import countries into the Kumasi Metropolis of Ghana. DOI: 10.20944/preprints202012.0254.v1.

Younes O, Ibrahim H, Hassan M, Amin R. 2019. Demonstration of some foodborne pathogens in different meat products: a comparison between conventional and innovative methods. Benha Vet Med J 36 (2): 219-228. DOI: 10.21608/BVMJ.2019.12557.1008.

Zhao X, Lin CW, Wang J, Oh DH. 2014. Advances in rapid detection methods for foodborne pathogens. J Microbiol Biotechnol 24 (3): 297-312. DOI: 10.4014/jmb.1310.10013.