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Microbial contamination of meat at a low temperature storage: A review

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ABSTRACT

Beef, mutton, lamb, and camel are all high-quality protein sources in Egypt and around the world. Red meat with a protein content of about 20%, a high moisture content (75%), fat (5.2%), carbohydrate (1.5%), vitamins such as vitamin B complex, and minerals such as iron, zinc, calcium, and phosphorus are important in human nutrition because they can meet a portion of man's daily needs for these nutrients. Low temperature storage of meat either at chilling or freezing conditions is very popular worldwide for the purposes of meat security, meat transportation, and overseas trade. However, the microbial quality of the meat at low temperature storage represents a challenging task for both the food safety and public health sectors. This review threw the light on the microbial status of chilled and frozen meat with a particular focus on the contamination of meat with *Pseudomonas* spp.

Introduction

Red meat, such as beef, mutton, lamb, and camel, is a major source of high-quality protein in Egypt and around the world. Red meat with a protein content of about 20%, a high moisture content (75%), fat (5.2%), carbohydrate (1.5%), vitamins such as vitamin B complex, and minerals such as iron, zinc, calcium, and phosphorus play an important role in human nutrition because it can meet a portion of man's daily needs for these nutrients (Klobukowski *et al*., 2002; Sallam and Morshdy, 2008; Elabbasy *et al*., 2021, Darwish *et al*., 2023; Morshdy *et al*., 2023). Red meat is high in important amino acids as arginine, histidine, leucine, isoleucine, lysine, methionine, riboflavin, threonine, and valine (Löest *et al*., 1997).

Microbial contamination of carcass surfaces is inherent during the process of transforming living animals into meat. While the majority of the microflora transferred to carcasses during the slaughtering process is non-pathogenic, pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter* spp., and *L. monocytogenes* may be present, making this one of the most critical quality and safety issues confronting the meat industry. Furthermore, with the increase in global trade and consumer awareness of the sanitary quality of meat in recent years, international emphasis has been focused on strategies to improve the microbiological quality and safety of foods. However, in order to assess the efficacy of any intervention strategy, the microbiological status of the environment must be known (Biswas *et al*., 2008).

Meat has been discovered to be an important means of transmitting foodborne diseases to humans all over the world. Microbial meat contamination can cause food-borne diseases in humans. Meat microbial loads must be investigated in order to limit the potential of microbial meat contaminants causing consumer illness. There has been a dramatic increase in public awareness of foodborne infections, particularly those associated with meat and animal products, in recent years. The major way that meat can spread infections and diseases is during preparation or ingestion by the customer. Furthermore, slaughterhouse techniques such as peeling, evisceration, and chilling are commonly responsible for meat contamination by foodborne microorganisms. As a result, veterinary public health officials must conduct constant microbiological surveillance of slaughtered animals to minimize microbial contamination (Ncoko *et al*., 2020; Darwish *et al*., 2022).

Temperature is a major factor that influences microbial growth. Most of the microorganisms are classified as mesophilic, some bacteria like low temperature and classified as psychrophilic bacteria, while bacteria that prefer higher temperatures are categorized as thermophiles. As meat industry is progressing and the trade of slaughtered animals is expanding so low temperature preservation is the most preferred method to keep the microbial growth at the minimum. Low temperature storage can be divided into chilling of meat at temperatures ranged between 1 to 7°C, which is more suitable for short term preservation. Freezing is the second type of the low temperature preservation at temperatures from zero to below. The latter is the most common method used for the preservation of meat. In the meat sector, freezing is used for long-term preservation and increases food security by drastically reducing bacterial deterioration. At the same time, freezing has a substantial impact on meat quality, such as moisture loss and protein denaturation (Muela *et al*., 2010; Leygonie *et al*., 2012; Morshdy *et al*., 2022).

The primary spoiling organisms in aerobically stored chilled beef are psychrotrophic *Pseudomonas* species. These species have various meta-

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bolic features that let them resist the extreme environmental conditions of low temperature storage and compete with other psychrotrophic rotting microbes. *Pseudomonas fragi* is the most commonly detected psychrotrophic spoilage *Pseudomonas* species on meat, including beef, poultry, hog, lamb, and fish, globally. Under the chilled temperature conditions employed in the meat industry, *Pseudomonas fragi* frequently forms biofilms on meat. When biofilms interact with meat exudate, slime forms, which is a major quality fault that causes consumer rejection of meat (Wickramasinghe *et al*., 2021).

This review threw the light on the microbial quality of the meat at low temperature, with a particular symphysis on meat contamination with Psuedomonas spp. at cold storage.

Microbial contamination of meat at low temperature

To characterize rates of microbial growth and pH changes in commercially prepared items of normal storage quality, the microbial ecology of fresh vacuum-packed pork slices was studied during storage at -1.5 degrees C for up to 45 days by Holley *et al*. (2004). Pork loins in commercial distribution with smell issues were also investigated in order to identify a possible cause of the defects and avert future difficulties. Furthermore, after 25 days of storage at -1.5 degrees Celsius, the microbiological profiles of pork slices from two plants were compared to discover plausible reasons for discrepancies in the storage life of product from the plants. The impact of a modification in sanitation processes on the microbial populations of 25-day-old items were also investigated. Microbial growth in different packages progressed at varied rates with normal product, showing disparities in baseline levels of bacterial contamination. All samples in the investigation survived 8 weeks with no visible organoleptic change and contained 5.8+/-1.2 log bacteria cm (-2) (mean+/-S.D.). The flora of odour-defective loins was dominated by lactic acid bacteria (LAB) and carnobacteria, while substantial proportions of *Enterobacteriaceae* were found 35 days after packaging. The sulfide-putrid smell of these damaged items was most likely caused by *Aeromonas* spp. and *Shewanella* spp., but *Enterobacteriaceae* and lactic acid bacteria could also have contributed to spoilage. A comparison of microbial groups present in 16 additional cuts, half from each of two commercial plants, held for 25 days at -1.5 degrees C, revealed that bigger groups were present. Larger proportions of *Enterobacteriaceae* were found in samples from a plant that was having problems obtaining the necessary storage life. Additional bacterial samples from 12 slices supplied by the latter plant and held for 25 days at -1.5 degrees C yielded few *Enterobacteriaceae*, *Aeromonas*, or *Shewanella*. The use of an acid sanitizer in factory cleaning could help reduce alkali-tolerant bacteria like *Aeromonas* or *Shewanella*, which can infect pig cuts and ruin vacuum-packaged product. The fraction of *Enterobacteriaceae* in bacteria populations on fresh pork held for 25 days at -1.5 degrees Celsius may be a good measure of plant sanitation efficacy.

Biswas *et al*. (2008) identified potential pre-slaughter and processing sources of psychrophilic and psychrotolerant clostridia producing vacuum-packed chilled meat deterioration. *Clostridium gasigenes*, *Clostridium estertheticum*, *Clostridium algidicarnis*, and *Clostridium putrefaciens* were detected in 357 samples collected from ten slaughter stock supply farms, slaughter stock, two lamb-processing plants, their environments, dressed carcasses, and final vacuum-packed meat stored at -0.5 degrees C for 5(1/2) weeks using molecular methods based on polymerase chain reaction (PCR) amplification of specific 16 *Clostridium gasigenes*, *C. estertheticum*, and *C. algidicarnis*/*C. putrefaciens* were often discovered in farm, faecal, fleece, and processing ambient samples obtained prior to fleece removal, however all of these microorganisms were detected in only 4 out of 26 cooling water samples. One of 42 boning room ambient samples were positive for *C. gasigenes* and *C. estertheticum*, but 25 of 42 tested positive for *C. algidicarnis*/*C. putrefaciens*. Almost all of the 31 faecal samples tested positive for *C. gasigenes* and *C. estertheticum*, however only two of them tested positive for *C. algidicarnis* and/or *C. putrefaciens*. Clostridial species were commonly found on cooled dressed carcasses throughout our experiment.

The microbial composition of vacuum-packed chilled meat was studied. The number of microbial counts grew during the 21-day storage period as the beef deteriorated. For the study of microbial diversity from vacuum packed pork during refrigerated storage, a total of 28,216 bacterial sequences were collected. More than 200 bacterial taxa from eighteen phyla were identified, and the majority of them are believed to be related with contamination during slaughtering and subsequent meat handling via fecal, air, and/or water. During storage, microbial populations altered dramatically, with the seventh day being a significant time point for microbial diversity. The key components that may be related with meat decomposition were *Micrococcaceae, Flavobacteriaceae, Enterobacteriaceae, Lactobacillaceae*, and *Carnobacteriaceae*. Although the possible influence of discovered bacteria on meat hygiene and/or safety is uncertain, proper decontamination of the entire chain is always vital for the meat business to ensure meat safety and increase fresh meat shelf-life (Zhao *et al*., 2015).

Pseudomonas fragi is the most common bacterium species found in ruined aerobically preserved chilled meat around the world. Under the chilled temperature conditions employed in the meat industry, it quickly forms biofilms on meat. Slime formation on meat is caused by biofilm growth, which is a serious quality problem. RNA sequencing was performed for the main stages of the P. fragi 1793 biofilm to understand the genetic regulation that allows P. fragi to survive under chilled conditions used in the meat industry, as well as to obtain an overview of this organism's transcriptomic behaviour when grown as biofilms. At each stage of the biofilm cycle, RNA was extracted: initiation, maturation, and dispersal. At the same time, fluorescence staining was used to assess biofilm formation. The RNA sequencing data were validated using qRT-PCR on the twelve genes that were most significantly up and down regulated at each stage. During biofilm maturation, differential expression analysis identified 332 significantly upregulated genes and 37 significantly downregulated genes compared to initiation. A comparison of gene expression at biofilm dispersal revealed 658 upregulated and 275 downregulated genes compared to initiation. Genes encoding flp family type IVb pilin, ribosome modulation factor, and creatininase were the most upregulated during biofilm maturation and dispersal, while genes encoding iron uptake systems such as TonB-dependent siderophore receptor and taurine transport were significantly down regulated. Protein synthesis and cellular proliferation stop once the biofilm population reaches its maximum (Wickramasinghe *et al*., 2021).

Pseudomonas aeruginosa is a well-known bacterial infection that confers resistance to a wide range of antimicrobial treatments. The ability of *P. aeruginosa* to produce biofilms improves the drug resistance phenomenon. The rise of biofilm-forming *P. aeruginosa* MDR strains in livestock and food items is a major public health concern. Abbas *et al*. (2022) investigated 100 meat samples (50 each from chicken and mutton) that were obtained from several butcher shops and supermarkets, and *P. aeruginosa* was recovered using standard microbiological, biochemical, and molecular techniques. The Kirby Bauer method was used to detect the resistance profile against various antibiotics, while the microtiter plate assay was used to detect biofilm development. Polymerase chain reaction was used to identify the biofilm associated gene (pslA) and the extended spectrum beta lactamase (ESBL) genes. *P. aeruginosa* was recovered from 24% of the meat samples tested, including 14/50 (28%) from chicken and 10/50 (20%) from mutton. AmoxicillinClavulanic acid and Ceftriaxone had the highest resistance (100%), followed by Aztreonam, Ticracillin (95.83%), and Ciprofloxacin (91.67%). 22 (91.66%) of 24 isolated *P. aeruginosa* were found to be MDR. Furthermore, 19 (86.36%) of the 22 MDR isolates were confirmed to be biofilm forming *P. aeruginosa*, and all of them tested positive for the biofilm encoding gene (pslA). Furthermore, 9 (40.90%) of the MDR isolates contained ESBL genes, including 6 bla_{CTX-M}

and 03 bla_{TEM}, but none of the isolates contained bla_{NDM} or bla_{OXA} genes.

In Iraq, from November 2020 to April 2021, one hundred frozen meat and chicken product samples were collected at random from commercial marketplaces in Misan Governorate, Iraq, and included: (burger - sausage - kebab - shawarma - minced meat) of beef and (chest-thigh-liver-burger-kebab) of chicken. Aerobic plate count findings revealed that all meat and poultry products were contaminated with bacteria, however foreign products were more polluted than domestic products. Using normal and standard bacteriological assays, we isolated and identified eighteen bacterial species. Gram-negative bacteria were found in 52% of beef products, while Gram-positive bacteria were found in 48%. Gram-positive bacteria predominated in chicken samples (59%) while Gram-negative bacteria predominated (41%). Using a universal 16S rRNA primer that yielded a 1500 bp amplification product, monoplex PCR was used to identify eighteen bacterial species. Nucleotide sequences were analysed at the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (Nucleotide BLAST). *Aeromonas veronii*, *Pseudomonas plecoglossicida*, *Acinetobacter lwofii*, *Acinetobacter lwofii*, *Aeromonas veronii*, *Klebsiella pneumoniae*, *Pseudomonas japonica, Pseudomonas songnensis*, *Klebsiella pneumoniae* subsp. *ozaenae*, and *Psychrobacter* (Husain and Aziz, 2022).

Rood *et al*. (2022) examined if specific microbes influence the spoiling rate of vacuum-packed (VP) lamb at low storage temperatures. A series of shelf-life challenge assays were used to assess the rotting potential of 13 representative microbes from the VP lamb spoilage community. Each strain was inoculated onto sterile (irradiated) and non-sterile (natural microbial community containing) VP lamb flesh. Meat quality was measured throughout time by sensory characteristics, bacterial growth, and pH. *Clostridium* spp. had the highest spoilage potential of all test species and had a significant effect on the spoilage rate of VP lamb (based on sensory assessment). *C. estertheticum* produced premature 'blown pack' deterioration in a community context, but the spoilage was delayed. *C. putrefaciens* and *C. algidicarnis* both induced premature spoiling of VP lamb in a community context. Except for *Carnobacterium divergens* and *Serratia* spp., which deteriorated meat prematurely when present in a community, all facultative anaerobes and *Pseudomonas* spp. tested were incapable of rotting meat independently or within a community. Overall, these findings suggest that *Clostridium* may be one of the key taxa responsible for the quicker rate of quality deterioration in chilled VP lamb compared to beef. This research can serve to inform options for shelf-life extension by focusing on organisms with a 'high' spoiling propensity, such as *Clostridium*.

Pseudomonas aeruginosa (*P. aeruginosa*) bacteria are considered the leading cause of food deterioration and food-borne illnesses at low temperature. Rezaloo *et al.* (2022) looked at the prevalence, antibiotic resistance characteristics, and distribution of virulence factors in *P. aeruginosa* bacteria isolated from meat and meat products. In Alborz province, Iran, 370 raw, frozen, and imported bovine meat samples, as well as various types of meat product samples, were gathered. Culture was used to identify *P. aeruginosa* bacteria. Antibiotic resistance in microorganisms was assessed using disc diffusion. PCR was also employed to determine virulence and antibiotic resistance genes. *P. aeruginosa* was found in 29 out of 370 (7.83%) of the samples. The highest distribution was seen in imported frozen bovine meat (20%), while banger (2%) had the lowest. *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria were shown to have high resistance rates to ampicillin (89.65%), penicillin (86.20%), tetracycline (82.75%), cefoxitin (37.93%), gentamicin (34.48%), and clindamycin (31.03%). The antibiotic resistance genes most commonly found were bla_{DHA} (93.10%), bla_{CTX-M} (83.65%), and bla_{SHV} (48.27%). The most common resistance genes were bla_{DHA} (93.10%), bla_{CTX-M} (83.65%), and bla_{SHV} (48.27%). *Exo*S (75.86%), *las*A (68.96%), *exo*U (58.62%), *las*B (51.72%), *plc*H (48.27%), and *alg*D (44.82%) were the most frequently found virulence genes. Meat and meat product samples may include *P. aeruginosa*, posing a significant risk to human ingestion. Nonetheless, additional research is required to discover additional epidemiological features of *P. aeruginosa* in meat and meat product samples.

In Egypt, Cairo and Giza, 170 random samples of imported frozen meat, imported frozen chicken, and locally frozen chicken were collected from various marketplaces. The samples were brought to the laboratory, where they were bacteriologically analysed to determine their hygienic status. Bacteriological investigation of the samples found that coliforms were present in 87, 80, and 72% of the imported frozen meat, imported frozen chicken, and locally frozen chicken, respectively. *E. coli* O was isolated from 8, 6, and 2.86% of locally frozen 158 chicken, imported frozen chicken, and imported frozen meat, respectively, and from 86 2 and 1.42% of imported frozen chicken and imported frozen meat, respectively. Furthermore, *E. coli* O 44 was recovered from only one sample from imported frozen chicken (2%). Other *E. coli* species were isolated from imported frozen meat, imported frozen chicken, and domestically frozen chicken, respectively. *E. coli* O:H and *Salmonella* species were not isolated from any of the samples tested. *Proteus mirabilis*, 157 7 *Pseudomonas*, Klebsiella, Enterobacter, and Providencia stuarti were isolated from the studied samples at various rates. The public health risk posed by these bacteria was examined, as well as the proposed actions to improve the sanitary quality status of imported frozen beef and poultry, whether imported or locally produced (Elnawawi *et al*., 2012). While, Ibrahim *et al*. (2016) reported that at various production dates, 90 random samples of frozen American, Brazilian, and Indian meat (30 of each) were obtained from various retail shops and supermarkets in EL-Menofeya Governorate. The collected samples were analysed bacteriologically and utilising PCR techniques to detect *Pseudomonas* species, particularly *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas cepacia*, *Pseudomonas fluorescence*, *Pseudomonas proteolytica*, *Pseudomonas versicularis*, *Pseudomonas fragi*, *Pseudomonas putida Ps. fragi*, *Ps. Putida*, *Ps. orientalis*, and *Ps. stutzeri* were isolated from 2(6.67%), 5 (16.67%), 1 (3.33%), 14 (46.67%), 8 (26.67%), 3(10%), 10 (33.33%), 6(20%), 1 (3.33%), and zero for frozen American meat samples, 3 (10%) , 8 (26.67%) , 1 (3.33%) , 19 (63.33%),5(16.67%), 7 (23.33%), 13(43.33%), 9 (30%) 3(10%) , 2(6.67%) , 1(6.67%) and zero for *Ps. orientalis* one *Ps. stutzeri* , for frozen Brazilian meat samples, 6(20%),9(30%),4 (13.33%),25(83.33%),6 (20%), 11(36.67),19(63.33%) , 13(43.33%), 4(13.33%), 3(10%), 3(10%), 1(3.33%) and 2(6.67%) for frozen Indian meat samples, respectively. Regarding *Ps. aeruginosa*, the total number and percentage of *Ps. aeruginosa* were 2(6.67%), 3(10%), and 6(20%) for American, Brazilian, and Indian frozen meat, respectively, with a total result of (12.22%). All conventionally investigated samples yielded positive results to *Pseudomonas* species utilizing the PCR methodology. *Ps. aeruginosa*, on the other hand, was discovered in one sample of Indian meat by traditional methods but not by PCR. Moreover, Khalafallah *et al.* (2020) mentioned that imported frozen meat is frequently more contaminated with several bacterial species than home butchered meat. Furthermore, the chemical and enzymatic processes of meat promote deterioration. One hundred samples of imported Brazilian frozen meat (50 frozen cubic meat and 50 minced meat) were gathered from different supermarkets in El-Menoufia Governorate, Egypt, and bacteriologically analysed for the identification of *Pseudomonas* species. *Pseudomonas* species were found in (35/50) of the frozen cubic beef. In contrast, the presence of such organisms was found in 80% (40/50) of the frozen minced beef samples tested. The psychrotrophic bacterial count in the frozen cubic meat analysed ranged from $6x10^2$ to $1.9x10^5$, with a mean value of $2.24x10^4$ cfu/g. Furthermore, the psychrotrophic bacterial count in frozen minced meat ranged from $7x10^2$ to $9x10^5$, with a mean value of $1.7x10^5$ cfu/g.The prevalence of recognised *Pseudomonas* species (number and percentages) detected in frozen meat samples represented by *P. aeruginosa*, *Ps. fluorescence, Ps. diminuta, Ps putida,* and *Ps. fragi* were 15 (30%), 40 (80%), 8 (16%), 5 (10%), and 4 (8%), respectively. The incidence of identified *Ps. aeruginosa*, *Ps. fluorescence, Ps. diminuta*, *Ps. Putida*, and *Ps. fragi* in minced meat samples was 20(40%), 45(90%), 5(10%), 7(14%), and 8(16%), respectively. Besides, Farghaly *et*

al. (2022) demonstrated that contamination of meat and meat products with pathogenic and spoilage microorganisms is one of the most serious challenges confronting the meat industry, resulting in a variety of human health issues and economic losses. The purpose of this study was to find *Pseudomonas* spp., specifically *Pseudomonas aeruginosa* (*Ps. aeruginosa*), in several processed and ready-to-eat beef products in Sohag governorate. Over a 12-month period, from November 2020 to October 2021, 200 random meat product samples were acquired from several markets in Sohag governorate, Egypt, including minced beef meat, luncheon, burger and sausage (50 of each). *Pseudomonas* spp. was found in 32 (15%) of the meat products tested utilizing colony morphology on Cetrimide agar, with the percentages being 30%, 18%, 6%, and 10% in minced beef meat, luncheon burger, and sausage, respectively. The PCR results revealed that only 8/12 (66.7%) of the suspected isolates encoded the 16S rDNA gene of *P. aeruginosa* with an incidence of 4% of the total examined samples, 4 (50%) of which were detected in the minced beef meat samples, 2 (25%) in the sausage samples and only 1 (12.5%) in the luncheon and burger samples.

Conclusion

This review confirmed the potential contamination of meat at cold storage with a vast array of food poisoning bacteria, particularly, *Pseudomonas* spp. Therefore, continuous screening and monitoring of microbial contamination of meat and meat products at cold storage is of a particular importance for the sake of human health and food safety.

Conflict of interest

The authors declare that they have no conflict of interest.

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