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Microbial Status of the Retailed Meat Products in Sharkia Governorate, Egypt

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Abstract

Ensuring food safety and strict hygienic practices adopted across the meat industry's whole supply chain is a primary responsibility of the food safety sector. Monitoring the sanitary status of the retailed meat products is necessary to complete this task. Meat products including luncheon, sausage, burger, and minced meat retailed in Sharkia governorate, Egypt, were evaluated for hygiene indicators such as total bacterial counts (TBC), total coliforms count (TCC), total *Staphylococcus aureus* (TSC), and total mold counts (TMC). Furthermore, isolation of some pathogenic microorganisms such as *Salmonella* spp., and *Listeria* spp., was done. The results showed varying rates of microbial contamination in the examined meat products. Minced meat was generally the most contaminated with microorganisms. *Salmonella* spp., and *Listeria* spp., were isolated from the examined samples at varying rates. In conclusion, to produce meat products of excellent keeping quality, it is strongly advised to follow stringent hygiene procedures when handling meat from the time of slaughter and throughout all manufacture process.

KEYWORDS
Meat products, Hygiene indicators, Egypt, *Salmonella*, *Listeria*.

INTRODUCTION

Humans need animal-derived protein in large quantities to maintain proper bodily functions. Meat products such as minced meat, luncheon, sausage, and burger provide a part of the red meat consumed in Egypt and around the world. The essential fatty acids, zinc, calcium, and iron minerals, vitamin B complexes, and important amino acids needed to maintain optimal health can all be obtained from them. Nonetheless, a number of research (Morshdy *et al*., 2018; Elabbasy *et al*., 2021; Elshafie *et al*., 2022) have also connected red meat and meat products to a significant number of instances of food poisoning worldwide.

The presence of a diverse range of microorganisms in the atmosphere of meat processing facilities, slaughterhouses, and butcher shops is the main cause of microbial contamination of the surfaces of the meat products. This cross-contamination is caused by a number of processing procedures starting from the slaughtering processes, skinning, evisceration, deboning, carcass transportation, meat products manufacturing and distribution. Therefore, a wide range of bacteria, including those that can lead to food poisoning and harm public health, can contaminate meat products. Microbial contamination can occur from the animal itself, butcher knives, cutting boards, walls, floors, air, and water that come into contact with the body (Darwish *et al*., 2022; Morshdy *et al*., 2023b).

Total bacterial counts (TBC), total coliform counts (TCC), total *Staphylococcus aureus* counts (TSC), and total mold counts (TMC) are indicators of the hygienic practices of the plants that prepare and handle meat products. These are but a handful of instances. These placards clearly illustrate the hygiene precautions and handling methods that are in place (Darwish *et al*., 2018; Morshdy *et al*., 2023a).

Several foodborne pathogens can be transmitted to human via ingestion of contaminated meat and meat products such as *Salmonella* spp., *Listeria monocytogenes* and *S. aureus* (Morshdy *et al*., 2023b).

One of the food safety sector's main duties is to ensure that hygienic procedures are followed throughout the meat production process (Alsayeqh *et al*., 2021). This study was conducted to determine the microbiological status of the surfaces of the meat products (minced meat, sausage, luncheon, and burger) retailed in the Sharkia governorate, Egypt.

MATERIALS AND METHODS

Collection of samples

Eighty samples from retailed meat products including luncheon, burger, sausage, and minced meat (20 samples, each meat product) were evenly and randomly collected from Sharkia Governorate, Egypt during March 2022 to February 2023. Samples were collected and directly transported cooled to the Laboratory of Meat Hygiene, and Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Microbiological examinations

Fifty grams from each of the samples under investigation

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were homogenized in a sterile 0.9% saline solution (450 mL). Each homogenate sample was then preceded to decimal serial dilutions (APHA, 2001). One milliliter of the intended dilution was used for each Petri dish or test tube utilized in the next experiments.

Total Bacterial Counts (TBC)

To estimate the overall quantity of bacteria, the APHA approach was employed (APHA, 2001). A sterile, spotless Petri dish was pipetted with one milliliter of each representative homogenate sample. Plate count agar (Difco Laboratories, Detroit, Michigan, USA) ranging from 12 to 15 milliliters was poured to each Petri dish, thoroughly mixed, and let to solidify before being inverted and incubated for 48 hours at 37°C. TBC was defined as plates with 25–250 pin-headed colonies.

TBC/g = average number of colonies × dilution reciprocal.

Colonies were counted and reported as $\log_{10}$ cfu/g.

Calculation of total coliform counts (TCC)

The total number of coliforms was estimated using the APHA method (APHA, 2001). To put it briefly, duplicate plates of an empty, previously sterilized Petri dish were filled with one milliliter of each prepared dilution, both initial and subsequent. Each Petri plate was then cooled to 45°C after adding 12–15 ml of violet, red bile glucose agar (Difco Laboratories, Detroit, Michigan, USA). The plates were incubated at 37°C for a whole day. Next, the colonies in pink and red were tallied. Colonies were counted and reported as $\log_{10}$ cfu/g.

Total count of Staphylococcus aureus (TSC)

The samples were then microbiologically processed using Baird Parker agar (Difco Laboratories, Detroit, Michigan, USA) in order to isolate *S. aureus* (APHA, 2001). *S. aureus* colonies were defined as black, glossy, convex colonies with a diameter of 1-1.5 mm and a distinct halo zone surrounding them. Gram's stain, biochemical tests such catalase, mannitol fermentation, coagulase, DNAs, and Voges-Proskauer (VP), as well as serological analysis, was used to investigate suspected colonies.

Reciprocal dilution factor x positive colonies equals the total *S. aureus* count. The number of colonies was given as $\log_{10}$ cfu/g.

Calculating the total number of molds

The total number of mold spores was determined by developing duplicate plates on Sabouraud's dextrose agar media (Oxoid, Basingstoke, UK) supplemented with 100 mg/L of chloramphenicol and incubating them at 25°C for 5-7 days in the dark. Throughout the incubation phase, the plates were examined daily to look for fungal growth. An estimate of the overall amount of mold was obtained by counting the cultured agar plates directly (APHA, 2001). Colonies were counted and reported as $\log_{10}$ cfu/g.

Isolation and identification of Salmonella spp.

Salmonella spp. were recovered and isolated in accordance with the guidelines provided by ISO 6579-1 (2017). To put it briefly, a milliliter (mL) of the prepared saline homogenate was poured onto nine milliliters (mL) of cold, sterile Rappaport Vassiliadis (RV) broth (Oxoid CM0669, UK) and incubated at 42°C for a full day. After that, a loopful was streaked onto Oxoid CM0469, UK's Xylose-Lysine Desoxycholate (XLD) agar, and it was incubated for 24 hours at 37°C. Five pure colonies—pink colonies with or without black centers-were purified once more using the same medium (XLD) and kept for later analysis in glycerol at -20°C. Following an inoculation into tryptic soy broth (TSB; Oxoid, Basingstoke, UK) and a 24-hour incubation period at 37°C, all of the stored colonies were revitalized once more. After that, XLD was streaked with a loopful of the turbid incubated broth, and it was incubated for 24 hours at 37 degrees Celsius. The biochemical tests that Kreig and Holt (1984) described were applied to the recovered isolates. Serological analysis was applied to the verified biochemical isolates, as per Kauffman's (1974).

Isolation and identification of Listeria monocytogenes

L. monocytogenes procedure was developed in accordance with Roberts *et al*. (1995). To summarize, 90 ml of *Listeria* enrichment broth base (CM862, Oxoid) with *Listeria* selective enrichment supplement (nalidixic acid, cyclohexemide, and acriflavine hydrochloride) (SR141, Oxoid) contained 10 ml of each incubated homogenate separately added. The mixture was then incubated at 37°C for 48 hours. Subsequently, a portion of the homogenate that had been incubated was collected and streaked onto Oxford agar plates using CM856, Oxoid selective medium. This was then supplemented with SR140, Oxoid selective supplement and incubated at 37°C for 48 hours. At least five green olive colonies with black zones around them were selected for additional purification using the same medium, and they were kept for later analysis at -80°C with 15% glycerol. After being refrigerated on tryptone soy agar (CM0131, Oxoid) supplemented with 0.6% yeast extract, the representative preserved colonies were cultured for 48 hours at 37°C. The reconstituted isolates underwent microscopic inspection to confirm that *Listeria* spp. were non-sporulating coccobacilli bacteria. Following this, the isolates underwent a battery of biochemical tests, including the catalase test, oxidase, sugar fermentation (D-glucose, L-rhamnose, xylose, and mannitol), and standard umbrella motility at 25°C. The hemolysin manufacturing process was applied to the isolates that showed positive results using Blood agar media (CM854, Oxoid) enriched with 5% sheep blood. Lastly, a *Listeria* latex agglutination kit (Oxoid, Basingstoke, Hampshire, England) was used on the anticipated isolates (Pagotto *et al*., 2001). Every step of the process was completed in compliance with the manufacturer's guidelines.

Statistical analysis

Every measurement was done twice, and the results are all shown as means ± SD. Bacterial counts were converted using base-10 logarithms of colony forming units per gram ($\log_{10}$ cfu/g). The statistical significance was evaluated using the Tukey-Kramer HSD test (JMP statistical tool; SAS Institute Inc., Cary, NC). To indicate statistical significance, P 0.05 was applied to all analyses.

RESULTS AND DISCUSSION

Eating food contaminated with pathogenic microbes or their toxins continues to be a leading cause of disease, hospitalization, and financial loss, even though public authorities and food operators are paying more attention to food hygiene and food safety (CDC, 2013). In addition, millions of individuals suffer from avoidable foodborne infections every year, which are a major economic burden and an increasing public health concern.

This study was done to investigate a serious food safety issue involving the regulation of sanitary standards of retailed meat

products. One of the primary goals of meat hygiene is to prevent cross-contamination between the food product and the meat handlers. The study's outcomes demonstrated that all tested samples contained mesophilic bacteria. After calculating the total bacterial count (TBC) (log_{10} cfu/g) in the acquired samples (Fig. 1), the mean TBC values were found to be 5.72±0.25, 5.24±0.36, 5.60±0.28, and 5.83±0.35 log_{10} cfu/g in the examined luncheon, burger, sausage, and minced meat samples, respectively. TBC was higher in the minced meat samples compared with the other examined samples. This could be explained by the unhygienic standards followed starting from the animal slaughter, dressing, evisceration, to the mincing process, and poor personal hygiene. Surfaces that came into contact with the carcass, especially the walls and cutting boards, also showed high TBC (Durmaz *et al.*, 2015). These findings are in line with those of Weistein (1991), who showed that inadequate personal hygiene was the cause of over 90% of hygienic problems in the food service industry. Furthermore, almost 25% of all food-borne infections can be attributed to improper hand washing alone, according to official statistics. Tambekar *et al.* (2008) indicated that inadequate hygiene in butcher shops increased contamination, which could have been brought on by unclean cutting boards, unclean walls, inappropriate handling, and a lack of knowledge about hygienic procedures. Furthermore, considering the existence of microorganisms in the environment, on animals, and on surfaces that come into contact with the carcass, Stoica *et al.* (2014) asserted that the presence of microbiological hazards in animal carcasses is unavoidable. These surfaces have the potential to house a multitude of microorganisms.

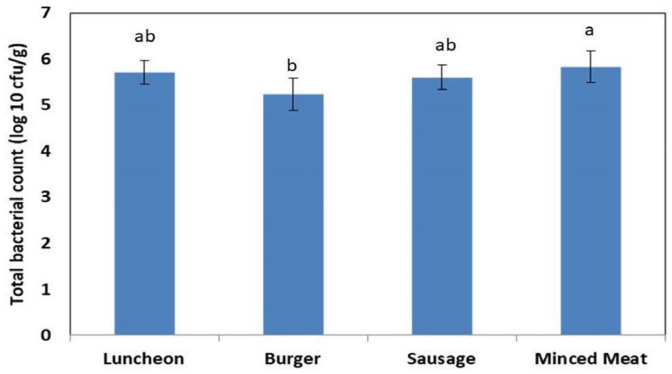

Fig. 1. Total bacterial count (TBC) of the examined meat products. Values are the averages ± standard deviations (Log_{10} cfu/g) of TBC of the examined luncheon, sausage, minced meat, and burger (n = 20). Columns carrying different letter are significantly different at $p<0.05$.

Total coliforms counts in the examined meat products were displayed in Fig. 2. With mean values of 3.14±0.20 log_{10} cfu/g, the data obtained clearly demonstrated that minced meat samples had the highest coliforms' counts when compared to other meat products. According to Darwish *et al.* (2015), coliform bacteria are significant markers of microbiological hygiene and highlight the significance of maintaining cleanliness in all stages of handling and preparing meat and meat products. The collected results demonstrated the presence of coliform bacteria on the investigated meat products. This result implies that such meat products were not prepared, handled, or manufactured in a hygienic way. The results we obtained agreed with those of Algabry *et al.* (2012), who discovered elevated levels of total coliform in cow carcasses and the surfaces they came into contact with at butcher shops in Alexandria, Egypt. The presence of the coliform group in meat has epidemiological significance since certain of its members are contagious and can result in serious diseases and food poisoning. Because of this and the fact that meat products can become recontaminated after processing. Coliforms can be used as a broad indicator of fecal contamination of meat products (ICMSF, 1996).

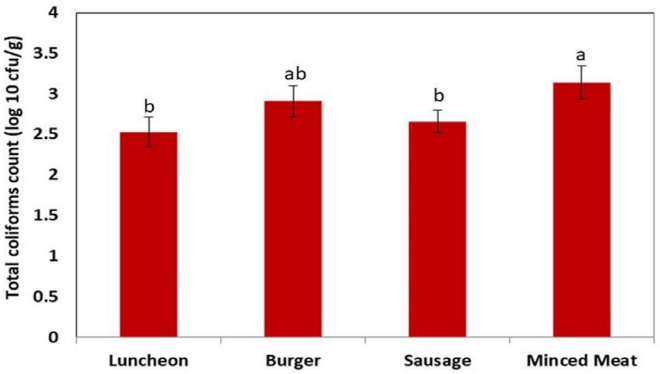

Fig. 2. Total coliforms count (TCC) of the examined meat products. Values are the averages ± standard deviations (Log_{10} cfu/g) of TEC of the examined luncheon, sausage, minced meat, and burger (n = 20). Columns carrying different letter are significantly different at $p<0.05$.

A total *S. aureus* count (log_{10} cfu/g) was estimated for the tested samples. The average total *S. aureus* counts in the examined sausage, minced meat, burger, and luncheon samples were 3.17±0.26, 2.99±0.26, 2.70±0.25, and 2.47±0.25 log_{10} cfu/g, respectively (Fig. 3). Because it frequently causes food poisoning outbreaks that have been connected to poor hygiene procedures, *S. aureus* contamination of the food supply is still an alarming issue on a global scale (CHP, 2011; Morshdy *et al.*, 2018). According to Morshdy *et al.* (2022, 2023a, b), food poisoning resulting from *S. aureus* is the third most prevalent cause of food-related illnesses worldwide. Aydin *et al.* (2011) stated that abrupt onset, vomiting, stomach cramps, and acute diarrhea with normal or below-normal body temperature are often indicative of food poisoning. Therefore, *S. aureus* infection can be utilized as a critical risk indicator for evaluating the food's safety and hygienic quality (Jyhshiun *et al.*, 2009) as well as a gauge of the hygienic conditions under which meat products are produced and handled (Potter, 2001).

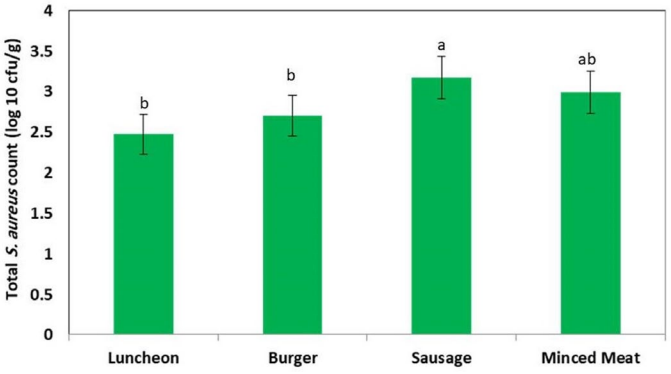

Fig. 3. Total *S. aureus* count (TSC) of the examined meat products. Values are the averages ± standard deviations (Log_{10} cfu/g) of TSC of the examined luncheon, sausage, minced meat, and burger (n = 20). Columns carrying different letter are significantly different at $p<0.05$.

The collected samples were subjected to an estimation of total mold counts (TMC) (log_{10} cfu/g). The obtained results indicated isolation of molds from 5 minced meat, 4 sausage, one luncheon, and one burger samples with prevalence rates of 25%, 20%, 5%, and 5%, respectively. The average TMC in the positive samples were 1.96±0.07, 1.67±0.04, 1.81, and 1.78, respectively (Fig. 4). The results of this study were in line with what we had previously published (Darwish *et al.*, 2016). Researchers have examined the frequency of meat contamination by different mold species in several countries, including Australia, Japan, Italy, and Spain (Hitokoto *et al.*, 1978; King *et al.*, 1986; Iacumin *et al.*, 2009; Martín-Sánchez *et al.*, 2011; Saleh *et al.*, 2020). Meat products

contaminated by fungi can decompose and release mycotoxins, which can harm human organs and cause cancer and liver disease (Darwish *et al.*, 2014).

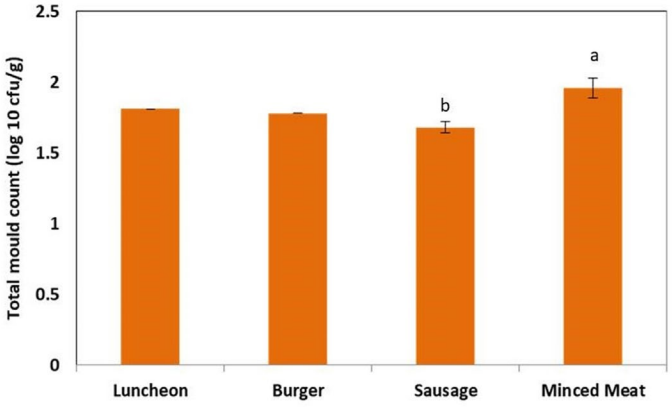

Fig. 4. Total mold count (TMC) of the examined meat products. Values are the averages ± standard deviations (Log_{10} cfu/g) of TMC of the examined luncheon, sausage, minced meat, and burger (n = 20). Columns carrying different letter are significantly different at $p<0.05$.

Salmonella spp. and *Listeria* spp. were isolated from the examined meat products. For *Salmonella* spp., the overall isolation rates were 30%, 25%, 20%, and 15% from the examined luncheon, sausage, minced meat, and burger, respectively (Fig. 5). For *Listeria* spp., it could be isolated at 20%, 5%, 5%, and 0% from the examined minced meat, sausage, luncheon, and burger, respectively (Fig. 6). Similarly, Arslan and Eyi (2010) isolated *Salmonella* spp. from ground beef retailed in Turkey at 21.3%. Besides, *Salmonella* spp. was also isolated from the retailed meat and meat products in Zaria, Nigeria at comparable levels (Tafida *et al.*, 2013). *Listeria* spp. was isolated at variable rates in different European countries varying from 0.96% in cooked meat retailed in Serbia to 58.3% in sausage sold in Slovakia (Kurpas *et al.*, 2018). Both *Listeria* spp., and *Salmonella* spp., are responsible for many hospitalizations worldwide (Alsayeqh *et al.*, 2021).

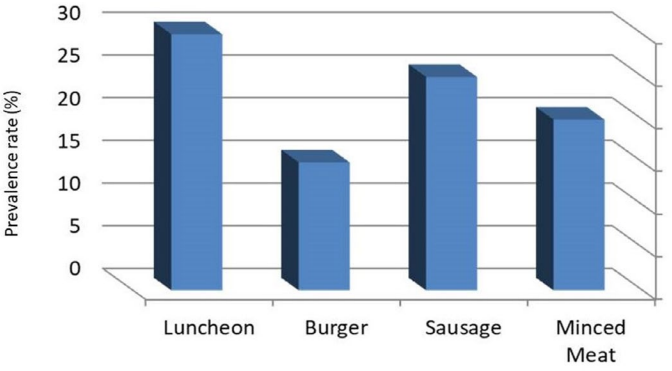

Fig. 5. Prevalence rate (%) of *Salmonella* spp., in the examined luncheon, sausage, minced meat, and burger samples.

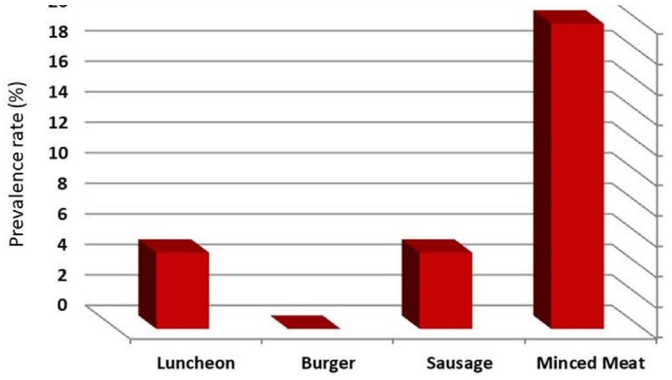

Fig. 6. Prevalence rate (%) of *Listeria* spp., in the examined luncheon, sausage, minced meat, and burger samples.

CONCLUSION

The results of this investigation showed that cross-contamination between animal corpses and the contacted surfaces is a well-researched phenomenon that needs to be considered when assessing the microbiological dangers. Therefore, we recommend that butcher shops periodically clean their machinery, use flowing water to wash their walls, and switch out their hardwood chopping boards for granite ones. There should be strict hygienic guidelines followed at every stage of handling animal carcasses.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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