

Hygienic Status of the Carcass Surfaces of Cattle, Buffalo, Sheep, and Camel Carcasses and their Contact Surfaces

Alaa Eldin M.A. Morshdy¹, Salah M.I. Mehrez¹, Ahmed E. Tharwat¹, Karima M.E. Abdallah¹, Wageh S. Darwish^{1*}, Ehab E. Nabawy², El-Shimaa M. Ali³

¹Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt.

²Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Egypt.

³Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt.

*Correspondence

Corresponding author: Wageh S. Darwish
E-mail address: wagehdarwish@gmail.com

Abstract

A main task for the food safety and animal hygiene sectors is to ensure safety and adoption of strict hygienic measures during the entire chain of the meat industry. To fulfill this task, continuous monitoring of the hygienic status of meat of different animal species and their contact surfaces is mandatory. In this study, the hygiene indicators including total bacterial counts (TBC), most probable number (MPN) of coliforms, total *Staphylococcus aureus* (TSC), and total mold and yeast counts (TMYC) of the surfaces of cattle, buffaloes, camel, and sheep retailed in Sharkia governorate Egypt were tested. Besides, such parameters were also investigated for the carcass contact surfaces including, the butcher hands, knives, cutting boards, and walls of the butcher shops. The obtained results indicated microbial contamination of the meat of different animals and their contact surfaces at variable rates. In general, cattle carcasses followed by that of the buffaloes had the highest microbial contamination. While walls and cutting boards showed the highest microbial contamination among the examined contact surfaces. In conclusion, adoption of strict hygienic measure during handling of the animal carcasses, sterilization of the carcass's contact surfaces is highly suggested to produce meat of high keeping quality.

KEYWORDS

Animal carcasses, Contact surfaces, Butcher shops, Hygiene indicators.

INTRODUCTION

Animal-derived protein is extremely important to humans in order to keep their bodies functioning normally. The main sources of red meat in Egypt and the rest of the globe are cattle, sheep, buffalo, and camels. They can supply humans with the vital fatty acids, zinc, calcium, and iron minerals, vitamin B complexes, and critical amino acids required to sustain good health. However, according to numerous studies (Sallam and Morshedy, 2008; Darwish *et al.*, 2014a; Elabbasy *et al.*, 2021; Elshafie *et al.*, 2022) red meat is also linked to many food poisoning cases over the world. Additionally, carcasses from cattle, buffalo, and sheep have high quantities of carotenoids and retinol (Darwish *et al.*, 2016a).

The primary source of microbial contamination of the surfaces of animal carcasses is the presence of a wide range of microorganisms in the atmosphere of meat processing factories, slaughterhouses, and butcher shops. Various processing steps, such as animal slaughter, skinning, evisceration, de-boning, corpse transportation, and distribution, result in this cross-contamination. As a result, raw meat can become infected with a variety of bacteria, including ones that can cause food poisoning and have a negative impact on public health. The animal itself, as well as things like butcher knives, cutting boards, walls, floors, air, and water that come into touch with the corpse, are sources of microbial contamination (Darwish *et al.*, 2022; Darwish and Thompson, 2023).

Microbial indicators for the hygienic practices of the meat-processing and handling plants include total bacterial

counts (TBC), most likely number (MPN) of coliforms, total *Staphylococcus aureus* counts (TSC), and total mould and yeast counts (TMYC). These are only a few examples. These signs paint a clear picture of the hygienic measures and handling techniques used while handling and processing corpses, which eventually affects the production of meat with a good preservation quality (Darwish *et al.*, 2018).

Assuring the hygienic practices used in all stages of meat processing is one of the sector's primary responsibilities (Alsayeqh *et al.*, 2021). In order to ascertain the microbiological status of the surfaces of the carcasses of cattle, sheep, buffalo, and camel sold in both rural and urban areas of the Sharkia governorate in Egypt, this study was carried out. Additionally, the cleanliness of the objects that came into contact with the carcass, such as butchers' hands, cutting boards, knives, and walls, was checked.

MATERIALS AND METHODS

Collection of samples

A total of 160 swab samples were evenly distributed and randomly obtained from animal carcasses (buffalo, cow) in the Sharkia district of Egypt. (n= 10 samples of each animal type from either rural or urban locales; sheep and camel, 20 samples each) from butcher shops in both urban and rural areas. Each swab sample was taken from the shoulder region of each carcass and represented a space area of 1x1 cm. In addition, 10 swab samples from butcher shops in both urban and rural areas were

also collected; each of the butcher hands, walls, knives, and cutting boards had a space area of one cm².

Microbiological examinations

Each sample under examination was removed with a sterile gauze swab dampened in a sterile 0.9% saline solution, and the removed material was then transferred to a sterile test tube containing 10 ml of the sterile 0.9% saline solution. The representative sample test tube was then applied after swabbing with a second dry swab. Following a vigorous shake, this dilution was determined to be 10⁻¹, and decimal dilutions were subsequently carried out (APHA, 2001). The following experiments employed 1 ml of the required dilution in each Petri dish or test tube.

Total Bacterial Counts (TBC)

The APHA technique was used to estimate the total number of bacteria (APHA, 2001). One mL of each swab representative tube was pipetted into a sterile, clean Petri dish. For each Petri dish, 12–15 mL of plate count agar (Difco Laboratories, Detroit, Michigan, USA) was added, mixed well, and then allowed to solidify before incubating inverted for 48 hours at 37°C. Plates with 25–250 pin-headed colonies were counted as TBC.

TBC/cm² = average No. of colonies × reciprocal of dilution
Counted colonies were expressed as log₁₀ cfu/cm².

Determination of most probable number of Coliforms (MPN)

The MPN method with three tubes, as recommended by APHA (2001), was used. In three test tubes with MacConkey broth and inverted Durham's tubes, one ml of each dilution was used to inoculate separately. The inoculated tubes were then incubated for 24-48 hours at 37 °C. Inverted Durham's tubes with positive results showed the formation of gas and acid (yellow color). The recommended tables were used to determine the number of coliforms that were most likely to exist.

Determination of the total mould and yeast counts

By cultivating duplicate plates on Sabouraud's dextrose agar media (Oxoid, Basingstoke, UK) supplemented with chloramphenicol 100 mg/L and incubating them at 25 °C for 5-7 days in the dark, it was possible to calculate the total number of mould and yeast. Every day during the incubation period, the plates were checked for fungal development. By directly counting the cultured agar plates, an estimate of the total amount of mould was derived (APHA, 2001). Counted colonies were expressed as log₁₀ cfu/cm².

Total *Staphylococcus aureus* count (TSC)

Following that the samples were processed microbiologically for the purpose of isolating *S. aureus* using Baird Parker agar (Difco Laboratories, Detroit, Michigan, USA) (APHA, 2001). Suspected colonies were sub-cultured on blood agar plates (Difco Laboratories, Detroit, Michigan, USA) and incubated for 24 hours at 37°C. Black, shiny convex colonies that were 1-1.5 mm in diameter and surrounded by a clear halo zone were counted as *S. aureus*. Suspected colonies were examined using Gram's stain, biochemical assays such as catalase, mannitol fermentation, coagulase, DNAs, and Voges-Proskauer (VP), and serologically (Quinn *et al.*, 2002). Total *S. aureus* count= Positive colonies x reciprocal dilution factor. Counted colonies were expressed as log₁₀ cfu/cm²

Statistical analysis

All data are presented as means SD, and each measurement was performed twice. Base 10 logarithms of colony forming units per cm² (Log₁₀ cfu/cm²) were used to convert bacterial counts. The Tukey-Kramer HSD test was used to assess statistical significance (JMP statistical package; SAS Institute Inc., Cary, NC). P < 0.05 was used in all analyses to denote statistical significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Even though public authorities and, in turn, food operators are paying more attention to food hygiene and food safety, eating foods contaminated with pathogenic microorganisms or their toxins continues to be a leading cause of illness, hospitalization, and financial loss (CDC, 2013). Additionally, millions of people experience preventable foodborne illnesses each year, which is considered a growing public health issue and has a significant impact on the economy.

Since one of the main responsibilities of meat hygiene is to prevent cross-contamination between the food product and the meat handlers, this study was conducted to investigate a significant food safety concern involving the regulation of hygienic practices in the butcher shops.

The obtained results of this study showed that mesophilic bacteria were present on all tested animal swabs and their contact surfaces (Table 1). In this investigation, the presence of coliform bacteria was found in the tested samples; the percentage of positive samples ranged from 10% (knives) to 80% (walls). Additionally, *S. aureus* was isolated and at variable rates that ranged from 20% (sheep surfaces, knives, and butcher hands) to 60% (walls). In this study, it was quite evident that animal corpses and their contact surfaces had been contaminated by fungi. From 20% to 100% of the samples tested positive for both yeast and mould contamination (Table 1).

Table 1. Microbial Contamination rates (%) of the examined carcasses and their contact surfaces.

	Mesophilic bacteria	Coliforms	<i>S. aureus</i>	Mold and Yeast
Buffaloes	100	50	40	50
Camel	100	40	30	50
Cattle	100	60	40	70
Sheep	100	30	20	50
Butcher hands	100	20	20	20
Cutting boards	100	30	40	40
Knives	100	10	20	20
Walls	100	80	60	100

Total bacterial count (TBC) (Log₁₀ cfu/cm²) was calculated in the samples that were obtained (Fig. 1), and the results showed that the mean TBC values in cattle samples sampled from urban and rural locations, respectively, were 4.6±0.22 and 5.8±0.33. Camels were much less contaminated than cattle and buffaloes, with values of 3.2±0.11 and 3.8±0.18 in samples taken from urban and rural locations, respectively. Sheep samples showed the lowest TBC when compared to other animal species in both urban (2.6±0.12) and rural (3.2±0.22) areas. In the second part of this study, where we evaluated TBC from the carcasses' contact surfaces (Fig. 2). The mean values of TBC in cutting boards were 5.5±0.33 and 6.2±0.18 Log₁₀ cfu/cm² from samples obtained from both urban and rural areas, respectively. Swab samples of walls from butcher shops in both urban and rural locations revealed

mean TBC values of 5.8 ± 0.22 and 6.50 ± 0.15 , respectively. The lowest TBC was found in swabs taken from knives and butcher hands, particularly those from urban regions. The data show that TBC was much greater in the carcasses of cattle and buffaloes than in those of camels and sheep, in both urban and rural areas. This might be explained by the unsanitary procedures used when handling the corpses in the rural butcher shops. The significant number of TBC pointed to the unsanitary circumstances present during the handling of the meat (Durmaz *et al.*, 2015). High TBC was also seen in the surfaces that came into contact with the carcass, particularly the walls and cutting boards. These results are consistent with Weistein (1991) who demonstrated that more than 90% of the sanitary issues in the food service business were the result of poor personal hygiene. Furthermore, according to official figures, incorrect hand washing alone is responsible for more than 25% of all food-borne illnesses. According to Tambekar *et al.* (2008), poor hygiene at butcher shops led to greater contamination, which may have been caused by dirty walls, unclean cutting boards, improper handling, and a lack of awareness of hygienic practices. Furthermore, according to Stoica *et al.* (2014), the presence of microbiological dangers in animal carcasses is inevitable given the presence of microorganisms in the environment, on animals, and on surfaces that come into touch with the carcass. These surfaces are susceptible to harboring a variety of germs.

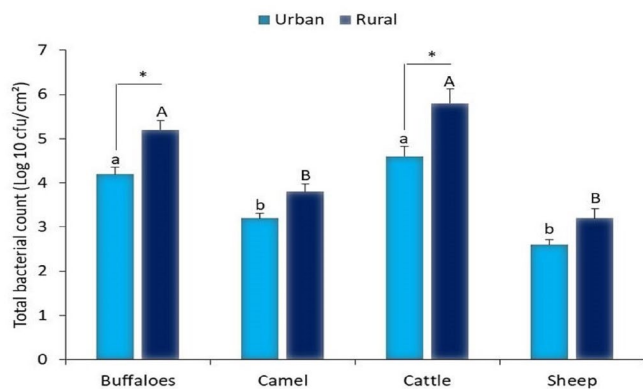


Fig. 1. Total bacterial count (TBC) of the examined carcasses' surfaces. Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TBC of cattle, buffaloes, camel and sheep carcasses. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

Figure 3 showed the MPN of coliforms in animal carcasses. The obtained data clearly showed that, when compared to other animal species, cattle samples, which were collected from both urban and rural locations, had the highest MPN of coliforms, with mean values of 3.40 ± 0.12 and 4.6 ± 0.18 Log_{10} MPN/cm², respectively. Swab samples taken from the surfaces of buffaloes in both urban and rural settings yielded these values of 3.2 ± 0.14 and 3.6 ± 0.2 Log_{10} MPN/cm², respectively. The MPN of coliforms was substantially ($p < 0.05$) lowest in sheep samples. It is evident from the results that the cutting boards and walls that were in contact with the animal carcasses had considerably ($p < 0.05$) higher MPNs of coliforms than the other surfaces (Fig. 4). Coliform bacteria are important microbiological hygienic indicators, emphasizing the importance of cleanliness throughout all processes of handling and preparing meat and meat products (Darwish *et al.* 2015). The acquired data showed that the examined animal carcasses and their contact surfaces contained coliform bacteria. This finding suggests that animal carcasses were not handled, prepared, or slaughtered in a hygienic manner. Our findings concurred with those made by Algabry *et al.* (2012), who found high total coli-

form counts in cattle carcasses and the surfaces they came into touch with at butcher shops in Alexandria, Egypt. Since some members of the coliform group are infectious and can cause serious infections and food poisoning, the presence of the group in meat has epidemiological significance. As a result, the coliform counts can be utilized as a general indicator of fecal contamination of meat due to insufficient processing and recontamination of meat after processing (ICMSF, 1996).

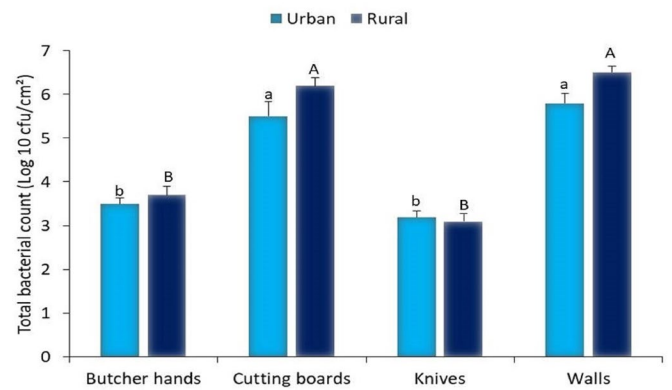


Fig. 2. Total bacterial count (TBC) of the examined carcasses' contact surfaces. Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TBC of butcher hands, cutting boards, knives, and walls. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

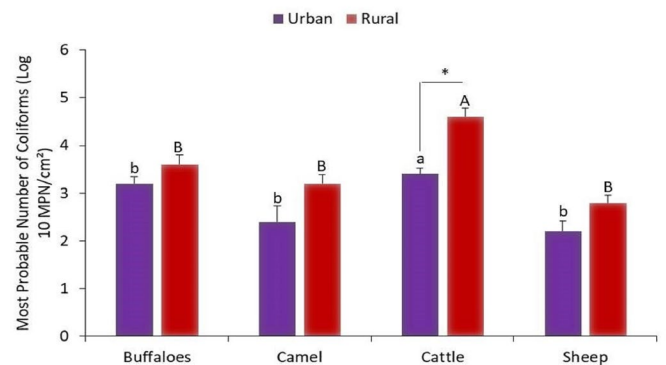


Fig. 3. Most probable number (MPN) of coliforms (MPN) of the examined carcasses' surfaces. Values are the averages \pm standard deviations (Log_{10} MPN/cm²) of MPN of coliforms of cattle, buffaloes, camel and sheep carcasses. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

In the samples that were tested, a total *S. aureus* count (Log_{10} cfu/cm²) was estimated. In cattle samples taken from both urban and rural locations, the mean total *S. aureus* counts were 3.7 ± 0.17 and 5.2 ± 0.15 Log_{10} cfu/cm², respectively. These values were 3.2 ± 0.22 and 4.82 ± 0.18 Log_{10} cfu/cm², respectively, in samples taken from buffaloes in both urban and rural locations. The mean total *S. aureus* counts in camel samples taken from both urban and rural areas were 3.4 ± 0.25 and 4.02 ± 0.22 Log_{10} cfu/cm², respectively, while the lowest values were found in sheep samples, 2.6 ± 0.16 and 3.6 ± 0.22 Log_{10} cfu/cm² from both urban and rural areas, respectively (Fig. 5). As shown in figure 6, swabs were taken from the contact surfaces of animal carcasses to determine the total *S. aureus* count (Log_{10} cfu/cm²). From both urban and rural locations, cutting boards had the highest overall *S. aureus* count (Log_{10} cfu/cm²), with mean values of 5.16 ± 0.22 and 5.80 ± 0.33 Log_{10} cfu/cm², respectively, while knives had the lowest *S. aureus* counts (Fig. 6). *Staphylococcus aureus* contamination

of the food supply is still a problem on a global scale since it is a common cause of food poisoning outbreaks that have been linked to improper hygiene practices (CHP, 2011; Morshdy et al., 2018).

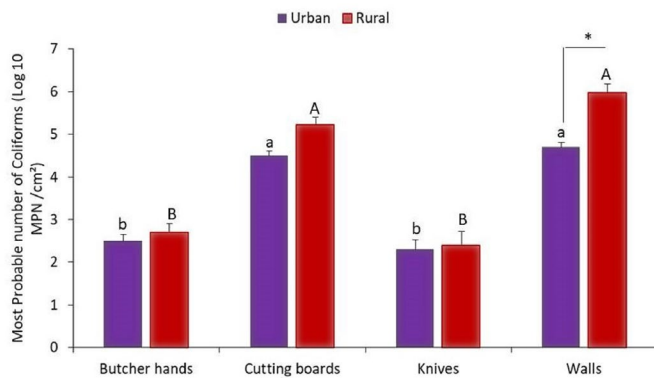


Fig. 4. Most probable number (MPN) of coliforms (MPN) of the examined carcasses' contact surfaces.

Values are the averages \pm standard deviations (Log_{10} MPN/cm²) of TBC of butcher hands, cutting boards, knives, and walls. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

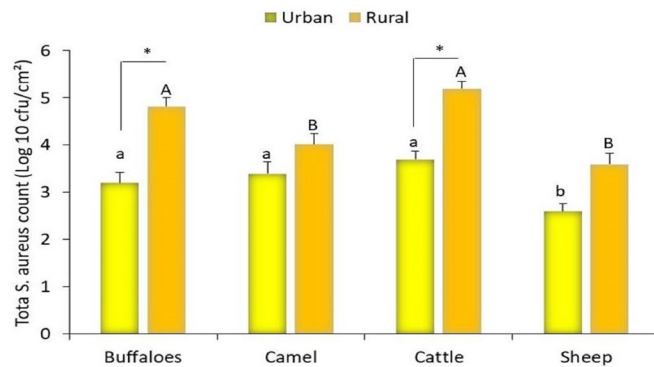


Fig. 5. Total *S. aureus* count (TSC) of the examined carcasses' surfaces.

Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TSC of cattle, buffaloes, camel and sheep carcasses. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

The findings of this investigation showed that samples of cattle and buffalo taken from rural areas had significantly higher levels of *S. aureus*. Swab tests of butcher hands, cutting boards, and walls, particularly those taken from rural regions, also revealed higher contamination levels. These findings support previously reported findings. Food poisoning caused by *S. aureus* is ranked as the third most common cause of food-related diseases globally (Morshdy et al., 2022). According to Aydin et al. (2011), food poisoning is frequently distinguished by its sudden onset, vomiting, cramping in the stomach, and acute diarrhoea with normal or below-normal body temperature.

As a result, *S. aureus* infection can be used to gauge the hygienic conditions under which meat is produced and handled (Potter, 2001), as well as a key risk indicator when assessing the food's safety and hygienic quality (Jyshiun et al., 2009).

Total mold and yeast counts (TMYC) (Log_{10} cfu/cm²) were estimated in the collected samples, the achieved results declared that the mean values of TMYC were 3.90 ± 0.24 and 4.80 ± 0.22 Log_{10} cfu/cm² in cattle samples collected from urban and rural areas respectively. These values were 3.56 ± 0.15 and 4.32 ± 0.23 Log_{10} cfu/cm² in the buffaloes' samples, respectively.

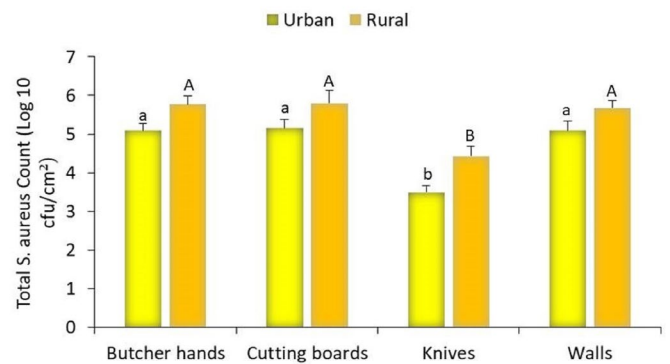


Fig. 6. Total *S. aureus* count (TSC) of the examined carcasses' contact surfaces. Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TSC of butcher hands, cutting boards, knives, and walls. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

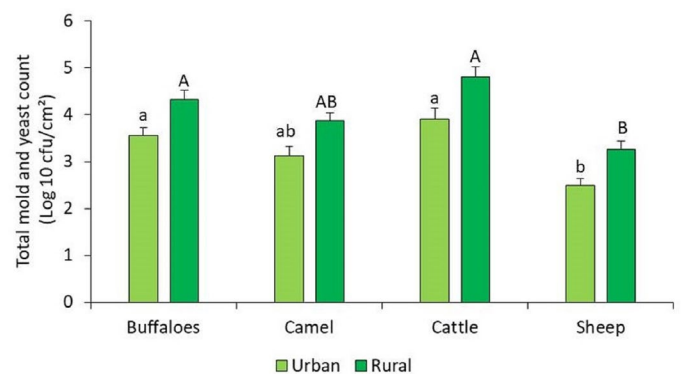


Fig. 7. Total mold and yeast count (TMYC) of the examined carcasses' surfaces. Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TMYC of cattle, buffaloes, camel and sheep carcasses. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

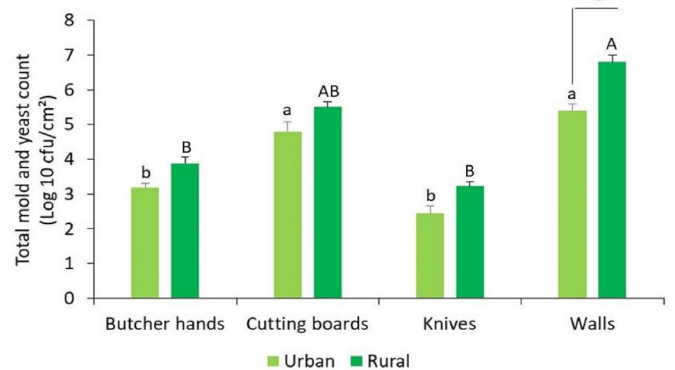


Fig. 8. Total mold and yeast count (TMYC) of the examined carcasses' contact surfaces. Values are the averages \pm standard deviations (Log_{10} cfu/cm²) of TMYC of butcher hands, cutting boards, knives, and walls. Columns carrying different letter A, B or a, b are significantly different at $p < 0.05$. * Mark indicates statistical difference among samples collected from rural and urban locations.

Camel samples were significantly lower than cattle and buffaloes samples. Sheep samples had the lowest TMYC compared with other animal species in both urban (2.5 ± 0.14) and rural (3.26 ± 0.18) areas, respectively (Fig. 7). The mean TMYC on cutting boards was 4.8 ± 0.28 and 5.50 ± 0.16 Log_{10} cfu/cm² from samples obtained from both urban and rural areas, respectively. We further evaluated TMYC from the carcasses' contact surfaces. Swab samples of walls from butcher shops in both urban and rural areas revealed mean TMYC levels of 5.4 ± 0.18 and 6.8 ± 0.2 Log_{10} cfu/cm², respectively. The lowest TMYC was seen in swabs taken from knives and butcher hands, particularly those from ur-

ban regions (Fig. 8). It was looked into how often mould or yeast was contaminated by fungi. The acquired results showed that carcasses and their contact surfaces had fungal contamination. Specifically butchered in rural butcher shops, cattle and buffaloes exhibited much higher total mould and yeast counts than camel and sheep carcasses. These findings corroborated the fungus-infested carcass-contact surfaces, particularly the walls and cutting boards. The findings of this investigation were consistent with those of our earlier publication (Darwish *et al.*, 2016b). In several parts of the world, including Australia, Japan, Italy, and Spain, researchers have looked into the prevalence of meat contamination by various mould species (Hitokoto *et al.*, 1978; King *et al.*, 1986; Iacumin *et al.*, 2009; Martín-Sánchez *et al.*, 2011; Saleh *et al.*, 2020). According to Darwish *et al.* (2014b), fungal contamination of meat can cause it to decay and produce mycotoxins, which can cause cancer, liver illness, and organ damage in humans. This study's findings on fungus contamination of animal corpses point to insufficient hygienic handling practices beginning at the abattoir level. The climate in slaughterhouses, stores, refrigerators, and butcher shops is particularly conducive to the formation of molds and yeasts, not only inside the meat but also on surfaces such as countertops and floors (Darwish and Thompson, 2023).

CONCLUSION

The findings of this study demonstrated that cross-contamination of animal carcasses and their contact surfaces is a well-documented phenomenon that should be taken into account when determining the microbiological risks. As a result, we advise butcher businesses to regularly cleanse their equipment, wash their walls with running water, and replace their hardwood cutting boards with granite. Every stage of handling animal carcasses should adhere to stringent hygiene standards.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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