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Prevalence of specific hygienic indicator bacteria in cattle slaughterhouses of different capacity in Egypt.

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ABSTRACT

Slaughtering should only take place in slaughterhouses supervised by veterinarians and with strict adherence to hygiene protocols to guarantee the production of high-quality meat. This study aimed to compare the hygiene of several slaughterhouses using total aerobic plate count (TAPC) and isolation of specific hygiene-indicating bacteria, such as Staphylococcus spp., Escherichia coli (*E. coli*), and Salmonella spp. 480 samples comprise swabs collected from various sources, such as knives (slaughter and skinning knifes), slaughterhouse buildings (wall, floor, and tap water samples), swabs from carcasses, and workers' shoes and hands. Based on our findings, the hygiene indicator microorganisms are negatively correlated with the biosecurity level. The TAPC was predominantly high in samples collected from slaughterhouse B, which had the lowest biosecurity score. The highest frequency of Staphylococcus spp., *E. coli*, and Salmonella spp. was (94.17%), (54.17%), and (5%), in slaughterhouses B, A, and C, respectively. PCR targeting the NUC gene was used for molecular confirmation of randomly selected Staphylococcus spp. as S. aureus. Only 60% of S. aureus isolates were positive for the biofilm-forming gene (BAP gene) in PCR. According to serological identification of *E. coli* isolates, the most popular *E. coli* serotypes in the cattle slaughterhouses were O44: K74, O142: K86, O119: K69, O164: K-, and O26: K60, while S. enterica sub-SP Salamae was the most frequent salmonella serotype. In conclusion, good personal hygiene and biosecurity measures serve as the primary safeguard against zoonotic infections in employees and bacterial colonization of livestock.

1. INTRODUCTION

Slaughterhouses are places where animals are slaughtered under the supervision of authorized and official veterinarians, with the primary purpose of producing meat suitable for human consumption (Mrdovic et al., 2017). Hygiene practices implemented within slaughterhouses are impacting both the quality of meat and the extent of contamination that can be attributed to various factors, such as contaminated working environments, surfaces, equipment, aerosols, and contaminated water (Laban et al., 2021). The assessment of the hygienic practices of slaughterhouses was carried out based on the following parameters: location, presence of a fence, source and availability of potable water, slaughter area and availability of basic facilities, ante and post-mortem facilities, waste disposal system, presence of toilets and bathrooms, protective clothing for workers, hygienic procedures of workers and the environment (Gali et al., 2020).

Implementing biosecurity protocols into the routine jobs of slaughterhouse employees can reduce the potential for zoonotic disease transmission and enhance food safety (Sesay et al., 2022). Scoring systems were developed to monitor the adherence to biosecurity protocols and their implementation (Van Steenwinkel et al., 2011). The possible score range is from zero, which indicates that the biosecurity measures stated are not being implemented at all, to one hundred, which indicates that the measures are being implemented in their entirety. The final overall score for biosecurity was the sum of the internal and external biosecurity scores, which were subdivided into different subcategory scores (Dewulf et al., 2018).

Total Aerobic Plate Count (TAPC) serves as a practical approach for monitoring food safety by indicating the total bacterial load in meat (Bersisa et al., 2019). The primary foodborne dangers associated with fresh meat include bacteria capable of causing human diseases such as *Staphylococcus* spp., *Escherichia coli* (*E. coli*) and *Salmonellae* spp. (Bersisa et al., 2019).

Staphylococcus spp. is responsible for causing food poisoning in humans when contaminated foods, such as meat, are ingested (Beyene et al., 2017) . *Staphylococcus aureus* is the primary cause of food poisoning due to its enterotoxins (Das et al., 2019). *E. coli* is responsible for causing colibacillosis in both humans and animals (Das et al., 2019). The detection of *E. coli* in food intended for human consumption shows poor hygiene during the processing and is indicative of fecal contamination (Atnafie et al., 2017). *Salmonella* spp. are among the most common food-borne pathogens worldwide and their infection is one of the major global public health problems (Takele et al., 2018).

This study aimed to assess the hygienic measures implemented in cattle slaughterhouses of different capacities across different localities in Egypt. This was achieved using total aerobic plate count (TAPC) and the detection of specific hygiene indicator bacteria, such as *Staphylococcus*

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spp., *E. coli, and Salmonella* spp., along with a biosecurity scoring system.

2. MATERIAL AND METHODS

2.1. Cattle slaughterhouses

The current investigation was conducted at four cattle slaughterhouses. The selection of slaughterhouses was based on slaughterhouse capacity, hygiene, and geographical location. Slaughterhouse A is in Qalyubia Governorate. Slaughterhouse B is in Menoufia Governorate. Slaughterhouse C is located in Qalyubia Governorate. Slaughterhouse D (the central traditional slaughterhouse) is in the Cairo Governorate. The basic information about slaughterhouses and applied hygienic measures under the study is listed in Table 1) which is prepared according to Ahsan et al., 2020).

2.2. Hygienic scoring of cattle slaughterhouses

Based on the parameters present in Table 1, a comprehensive biosecurity scoring system was used to assess hygienic practices in the slaughterhouses. These parameters included the slaughterhouse's surroundings, the state of its outer and inner buildings, the adherence to policy guidelines, the quality of water hygiene, visitor control, and workers' hygiene practices. The ultimate total score for slaughterhouse biosecurity was the sum of the different subcategory scores (Dewulf et al., 2018).

Calculation = Sum scores of total applied biosecurity measures × 100

Total full application of biosecurity measures

2.3. Sampling

A total of 480 samples and swabs were obtained from four slaughterhouses during three visits per each and five samples per visit were collected from each type of swab and sample. A set of sixty tap water samples were collected (15 per slaughterhouse), according to Soliman et al. (2022). A total of sixty slaughter knife swabs (15 per slaughterhouse) and sixty skinning knife swabs (15 per slaughterhouse) were collected, according to Abayneh et al. (2019). Additionally, a set of sixty hand swabs (15 per slaughterhouse) and sixty shoe swabs (15 per slaughterhouse) were collected, according to Abayneh et al. (2019). According to Soliman et al. (2022), sixty-floor and sixty-wall swabs (15 per slaughterhouse) were collected. Finally, a set of sixty carcass swabs (15 swabs per slaughterhouse) were collected, according to Tanih et al. (2015).

The swabs were immediately put into sterile tubes with 5 ml of buffered peptone water after collection and carried in an ice box to the laboratory to be examined. The collected samples were approved with an Ethical Approval Number (BUFVTM03-06-24).

2.4. Determination of Total Aerobic Plate Count (TAPC)

Total plate count agar was used for TAPC. The medium was autoclaved and maintained at 46 ºC. Samples were serially diluted, and an aliquot of 1 ml of each of the serial dilutions was transferred to the Petri dishes, where molten agar (15–20 ml) was poured on them. Plates were gently swirled to uniformly mix the sample and incubated at 37 ºC for 48 h. After incubation, TAPC was determined from appropriate plates (Ahmad et al., 2013).

2.5. Isolation and biochemical identification of specific hygienic indicator bacteria.

2.5.1. Isolationand biochemical identification of Staphylococcus spp.

Isolation of *Staphylococcus spp.* was carried out on Baird-Parker agar (BPA) supplemented with egg yolk telluride emulsion and incubated at 37 ˚C for 48 h, according to Hafez et al. (2022).

Biochemical identification of *Staphylococcus spp.* was carried out using the coagulase test (negative except for *S. aureus*) and the catalase test (positive), according to Hafez et al. (2022).

2.5.2. Isolation and biochemical identification of E. coli spp.

Isolation of *E. coli spp.* was carried out on Eosin Methylene Blue (EMB) agar and incubated at 37 °C for 24 h, according to Aidaros et al. (2022).

Biochemical identification of *E. coli spp.* was carried out using TSI (Triple Sugar Iron) test (Acid/Acid, Gas), Simmon Citrate test (negative), Urease (negative), and Indole test (positive), according to Tadese et al. (2021).

2.5.3. Isolation and biochemical identification of *Salmonella spp.*

Isolation of *Salmonella spp.* was carried out in the following steps: the samples were incubated aerobically in BPW at 37 ºC for 24 h. From the pre-enrichment tubes, 1 ml was inoculated into 9 ml Rappaport Vassiliadis (RV) broth and incubated aerobically at 42 ºC for 24 h. A loop full of selectively enriched broth was streaked separately onto Xylose Lysine Desoxycholate (XLD) agar and incubated at 37 ºC for 24 h, according to Aidaros et al. (2022).

Biochemical identification of *Salmonella spp.* was carried out using TSI test (alkaline/acid, positive H2S, and positive gas production), the urine test (negative), and the Indole test (negative), according to Sarker et al. (2021).

2.6. Molecular identification of Staphylococcus spp.

2.6.1. DNA extraction

DNA extraction from bacterial cultures was performed using the GF-1 Bacterial DNA Extraction Kit (Cat. No. GF-BA-100, Vivantis, Malaysia) following the manufacturer's recommendations.

2.6.2. Molecular identification using PCR

PCR was used to identify ten Staphylococcus spp. isolates from knives, slaughterhouse buildings, carcasses, and workers' shoes and hands. For identification of *S. aureus* targeting *NUC* gene, PCR reaction was performed using SimpliAmp™ Thermal Cycler (Cat. No. A24811, Applied Biosystems, USA) in a final volume of 25 µl reaction containing 12.5 µl of 2x COSMO PCR RED master mix (Cat. No. W1020300X, Willofort, UK), 0.5 µl (10 µM) of each primer and 1 µl of target DNA. The PCR products were separated by electrophoresis on 1.5% agarose gel and then photographed and analyzed by InGenius3 gel documentation system (Syngene, UK). The oligonucleotide primers for *NUC* gene were F,(5´-CTGGCATATGTATGGCAATTGTT-3´) and R, (5´-TATTGACCTGAATCAGCGTTGTCT-3´) with 664bp according to (Graber et al., 2007). The cycling conditions of primers during PCR for *NUC* gene were initial denaturation at 94 ˚C for 5 min, denaturation at 94 ˚C for 30 sec, annealing at 57 °C for 40 sec, and extension at 72 °C for 1 min. The number of cycles was 35 and the final extension was at 72 ˚C for 10 min.

2.6.3. Detection of biofilm formation genes.

All ten S. *aureus* isolates were screened for the presence of *BAP* (biofilm formation gene). The oligonucleotide primers for *BAP* gene were F, (5´-CCCTATATCGAAGGTGTAGAATTGCAC-3´) and R, (5´-GCTGTTGAAGTTAATACTGTACCTGC-3´) with 971 bp according to Cucarella et al. (2004). The cycling conditions of primers during PCR for *BAP* gene were initial denaturation at 94 ˚C for 5 min, denaturation at 94 ˚C for 30 sec, annealing at 57˚C for 30 sec, and extension at 72 ˚C for 45 sec. The number of cycles was 35 and the final extension was at 72 ˚C for 10 min.

2.7. Serological identification of E. coli spp. and Salmonella spp.

E. coli was serologically identified according to (Kok et al., 1996) and S*almonella spp.* was serologically identified according to Kauffman's White Scheme (Kauffman, 1974).

2.8. Statistical analysis:

The statistical analysis was carried out using Two-way ANOVA using SPSS, version. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design, according to Steel et al. (1997). Multiple comparisons were carried out using the Duncun test, and the significance level was set at *P*<

Table (1): The common parameters for assessment of hygienic practices of slaughterhouses

Parameter			Slaughterhouse (A)	Slaughterhouse (B)	Slaughterhouse (C)	Slaughterhouse (D)
Floor space			200 m²	150 m²	500 m²	25 hectares
Days of work			Two day / week	Daily	six days / week	six days / week
Capacity (Number of head / day)			4 head / day	3-15 head / day	20-25 head /day	32 – 46 head/ day
hygiene section observed at slaughterhouses	Use of foot bath and Wheel dip		-	-	-	-
	Separation of different species of animal slaughtered		-	-	√	√
	Separation between clean and unclean area		-	-	-	-
	Separation between Entrance and Exit area		-	-	-	√
	Ventilation system		Naturally ventilated	Naturally ventilated	Naturally ventilated	Naturally ventilated
	Worker's hygiene	Disinfection of hands before and after slaughtering	-	-	-	-
		Wearing gloves, head cover and white coat	-	-	-	-
		Wearing boots	√	√	√	√
		medical checkup	-	-	-	-
	Disinfection of slaughtering tools and knives before and after slaughter		-	-	-	-
	Disinfection of floor and wall		√	√	√	-
	Control of visitors		-	-	-	√
	Flies, Rodents and other animals' control		-	-	-	-
	Drainage system efficiency		-	-	√	√
	Daily disposal of waste		√	-	√	√
Facility section observed at slaughterhouses	Appropriateness of location of slaughterhouse		√	-	-	√
	Appropriate Distance from residential area		√	-	-	√
	Standard slaughterhouse design		-	-	√	√
	Enough space for future expansion		-	-	-	√
	Fence		√	√	√	√
	Availability of lairage		-	-	-	√
	Availability of condemnation room		√	√	√	√
	Availability of cold chain		-	-	-	-
	Availability of sufficient clean water		√	√	√	√
	Availability of toilets		√	√	√	√
Policy section observed at slaughterhouses	Documentation of numbers of animals slaughtered		√	√	√	√
	Performing ante and post -mortem examinations of animals		√	√	√	√
	Isolation of sick animals		√	√	√	√
	Resting of animals before slaughtering		-	-	-	√
	Use of Halal method of slaughter		√	√	√	√

(√) mean presence of parameter, (-) mean absent or not efficient parameter.

3. RESULTS

3.1. Biosecurity scoring

The biosecurity score was the highest in slaughterhouse D (66%), followed by slaughterhouse C (50%), slaughterhouse A (48.2%), and slaughterhouse B was the lowest (32.1%) (Figure 1).

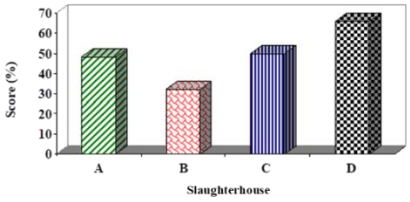

Figure (1): Biosecurity score (%) for each slaughterhouse

3.2. Total Aerobic Plate Count (TAPC) in the examined cattle slaughterhouses

As shown in Table 2, the samples collected from slaughterhouse B had the highest incidence of TAPC (log 5.51), followed by slaughterhouse D (log 4.88), slaughterhouse A (log 4.38), and slaughterhouse C (log 4.1). Additionally, significant variations were observed among the collected samples; hand and floor swabs contained the highest proportion of TAPC (log 5.4) per each, followed by skinning knife swabs (log 5.16), shoe swabs (log 4.82), carcass swabs (log 4.77), slaughter knife swabs (log 4.49), and wall swabs (log 4.03). Water samples contained the lowest percentage (log 3.69).

Table (2): Log number of total aerobic plate count (TAPC) in slaughterhouses.

Swab Sample type		Slaughterhouses				Mean of samples
		A	B	C	D	
Knife	Slaughter	4.42±1.08^{abB}	5.93±0.79^{aA}	3.80±0.58^{bcB}	3.82±0.42^{dB}	4.49±0.42^{abc}
	Skinning	4.84±1.18^{aB}	5.74±0.64^{aA}	4.26±0.03^{abB}	5.78±0.61^{abA}	5.16±0.37^{a}
Slaughterhouse building	Wall	2.59±0.06^{cC}	4.76±0.16^{bcB}	3.27±0.48^{cC}	5.48±0.59^{bA}	4.03±0.38^{bc}
	Floor	5.11±0.98^{aBC}	5.51±0.50^{abB}	4.50±0.12^{abC}	6.49±0.14^{aA}	5.40±0.32^{a}
	Tap water	5.02±1.27^{aA}	3.99±0.66^{cB}	3.24±0.58^{cC}	2.50±0.01^{eD}	3.69±0.43^{c}
Workers	Hands	4.87±1.20^{aB}	6.32±0.14^{aA}	4.94±0.63^{aB}	5.48±0.62^{bB}	5.40±0.36^{a}
	Shoes	4.36±1.04^{abC}	6.10±0.05^{aA}	3.80±0.56^{bcC}	5.02±0.56^{bcB}	4.82±0.38^{ab}
Carcass		3.84±0.68^{bC}	5.77±0.68^{aA}	4.98±0.55^{aB}	4.50±0.89^{cdBC}	4.77±0.37^{ab}
Mean of slaughterhouse		4.38±0.34^{BC}	5.51±0.22A	4.10±0.20^{C}	4.88±0.29^{AB}	

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

3.3. Prevalence of *Staphylococcus spp.*

The prevalence of isolated *Staphylococcus spp.* from various cattle slaughterhouses was (88.33 %). In different cattle slaughterhouses, the prevalence of Staphylococcus spp. varied significantly. The prevalence of *Staphylococcus spp.* was the highest in the samples collected from Slaughterhouse B (94.17 %) followed by Slaughterhouse A (91.67 %), Slaughterhouse D (85 %) and Slaughterhouse C (82.5%) (Table 3).

A substantial disparity in *staphylococcus* prevalence was observed among the samples collected from various slaughterhouses. The floor swabs contained the highest prevalence of *staphylococcus* (100%), while the water samples contained the lowest prevalence (43.33%) (Table 3).

The samples that are most contaminated with *Staphylococcus* in slaughterhouses (A) include the slaughter and skinning knife, floor, hands, shoes, and carcass samples. The samples that are most contaminated with *Staphylococcus* in the slaughterhouse (B) include the skinning knife, floor, wall, and hand swabs. In the slaughterhouse (C), the walls, floor, hands, shoes, and carcass samples are the most contaminated with Staphylococcus. The samples found to be most contaminated with *staphylococcus* in slaughterhouses (D) are the slaughter knife, floor, shoes, and carcass swabs, as illustrated in Table 3.

Table (3): The prevalence rate (%) of *Staphylococcus* in different cattle slaughterhouses

Swab Sample type		Slaughterhouses				Mean of samples
		A	B	C	D	
Knife	Slaughter	100.00^{aA}	93.33^{abA}	73.33^{bB}	100.00^{aA}	91.67^{a}
	Skinning	100.00^{aA}	100.00^{aA}	46.67^{cB}	93.33^{aA}	85.00^{a}
Slaughterhouse building	Wall	93.33^{aA}	100.00^{aA}	100.00^{aA}	93.33^{aA}	96.67^{a}
	Floor	100.00^{aA}	100.00^{aA}	100.00^{aA}	100.00^{aA}	100.00^{a}
	Tap water	40.00^{bB}	93.33^{abA}	40.00^{cB}	0.00^{bC}	43.33^{b}
Workers	Hands	100.00^{aA}	100.00^{aA}	100.00^{aA}	93.33^{aA}	98.33^{a}
	Shoes	100.00^{aA}	86.67^{abB}	100.00^{aA}	100.00^{aA}	96.67^{a}
Carcass		100.00^{aA}	80.00^{bB}	100.00^{aA}	100.00^{aA}	95.00^{a}
Mean of slaughterhouse		91.67^{AB}	94.17^{A}	82.50^{B}	85.00^{AB}	88.33

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

3.4. Prevalence of *E. coli spp.*

The prevalence of isolated *E. coli spp.* from various slaughterhouses was 39.58%. The prevalence of *E. coli spp.* significantly varied in different slaughterhouses. The prevalence of *E. coli spp.* was the highest in the samples collected from Slaughterhouse A (54.17%), followed by Slaughterhouse B (39.17%), Slaughterhouse C (35%) and Slaughterhouse D was the lowest (30%) (Table 4).

There is a statistically significant variation in the prevalence of *E. coli* in samples from several livestock slaughterhouses. As indicated in Table 4, the water samples had the lowest prevalence of *E. coli* at 10%, whereas the wall swabs had the highest percentage (60%).

Shoes and floor samples were the most contaminated samples collected in the slaughterhouse (A). Additionally, hand samples were the most contaminated in the slaughterhouse (B). However, the wall samples were the most contaminated in the slaughterhouse (C). The wall samples in the slaughterhouse (D) were the most contaminated, as shown in Table 4.

Table (4): Prevalence rate of (%) *E. coli spp.*in different slaughterhouses

Swab sample type		Slaughterhouses				Mean of samples
		A	B	C	D	
Knife	Slaughter	53.33^{bcA}	20.00^{cB}	20.00^{cdB}	26.67^{bcB}	30.00^{bcd}
	Skinning	40.00^{cA}	26.67^{bcAB}	13.33^{cdB}	26.67^{bcAB}	26.67^{cd}
Slaughterhouse building	Wall	53.33^{bcB}	46.67^{abB}	80.00^{aA}	60.00^{aB}	60.00^{a}
	Floor	80.00^{aA}	53.33^{aB}	46.67^{bB}	40.00^{abB}	55.00^{a}
	Tap water	0.00^{dB}	6.67^{cB}	6.67^{dB}	26.67^{bcA}	10.00^{d}
Workers	Hands	60.00^{abcAB}	66.67^{aA}	46.67^{bB}	26.67^{bcC}	50.00^{ab}
	Shoes	80.00^{aA}	46.67^{abB}	33.33^{bcC}	13.33^{cC}	43.33^{abc}
Carcass		66.67^{abA}	46.67^{abB}	33.33^{bcBC}	20.00^{bcC}	41.67^{abc}
Mean of slaughterhouses		54.17^{A}	39.17^{B}	35.00^{B}	30.00^{B}	39.58

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

3.5. Prevalence of *Salmonella spp.*

The prevalence of isolated *Salmonella spp.* from various cattle slaughterhouses was 2.29%. The prevalence rate of *Salmonella* spp. showed a highly significant difference between the different cattle slaughterhouses. The prevalence of isolated *Salmonella spp*. was the highest in slaughterhouse C (5%) followed by slaughterhouse B (2.5%) and slaughterhouse D (1.67 %), while slaughterhouse A was 0% (Table 5).

The prevalence of *Salmonella spp*. in the samples from several cattle slaughterhouses was found to be significantly different. Skinning knife swabs had the highest incidence of *Salmonella spp.* at 8.33%, followed by shoe swabs (5%), carcass swabs (3.33%) and floor swabs (1.67%). However, the results for Salmonella in the water, wall, slaughter knife, and hand samples were negative, as shown in Table 5.

Table (5): Prevalence rate (%) of *Salmonella spp*. in different slaughterhouses

Swab sample type		Slaughterhouses A	B	C	D	Mean of samples
Knife	Slaughter	0.00^{aA}	0.00^{cA}	0.00^{bA}	0.00^{bA}	0.00^{b}
	Skinning	0.00^{aC}	13.33^{aA}	13.33^{aA}	6.67^{aB}	8.33^{a}
Slaughterhouse building	Wall	0.00^{aA}	0.00^{cA}	0.00^{bA}	0.00^{bA}	0.00^{b}
	Floor	0.00^{aB}	6.67^{bA}	0.00^{bB}	0.00^{bB}	1.67^{b}
	Tap water	0.00^{aA}	0.00^{cA}	0.00^{bA}	0.00^{bA}	0.00^{b}
Workers	Hands	0.00^{aA}	0.00^{cA}	0.00^{bA}	0.00^{bA}	0.00^{b}
	Shoes	0.00^{aC}	0.00^{cC}	13.33^{aA}	6.67^{aB}	5.00^{ab}
Carcass		0.00^{aB}	0.00^{cB}	13.33^{aA}	0.00^{bB}	3.33^{b}
Mean of Slaughterhouse		0.00^{B}	2.50^{AB}	5.00^{A}	1.67^{B}	2.29

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B and C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

3.6. Molecular identification and confirmation of *Staphylococcus* isolates

Targeting the *NUC* gene with PCR, (10/10) (100%) of the tested *Staphylococcus spp*. that were isolated from workers, knives, slaughterhouse buildings, and carcasses were identified as *S. aureus* (Figure 2A). Additionally, as shown in Figure 2B, PCR targeting the *BAP* gene, which is responsible for biofilm formation, yielded positive results for (6/10) (60 %) of *S. aureus* isolates.

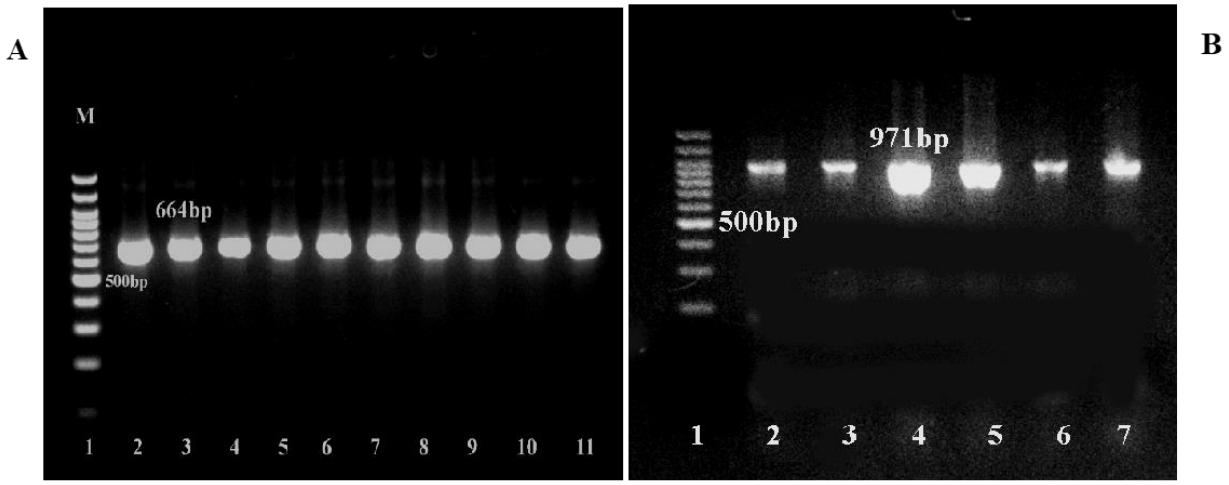

Figure (2) A: Agarose gel electrophoresis of PCR product amplified from *S.aureus NUC* gene (664 bp). Lane 1, 100 bp DNA Ladder; Lanes 2-11, positive samples. B: Agarose gel electrophoresis of PCR product amplified from *BAP* gene (971 bp). Lane 1, 100 bp DNA Ladder; Lanes 2-7, positive samples.

3.7. Serological identification and confirmation for *E. coli* isolates.

The most prevalent *E. coli* serotypes that were isolated from knives, slaughterhouse facilities, personnel, and carcasses from different slaughterhouses were O44:K74 (25%), O142:K86 (25%), O119:K69 (25%), O164: K- (12.5%), and O26: K60 (12.5%), as shown in Table 6.

3.8. Serological identification and confirmation for *Salmonella* isolates.

A strain of *S. enterica SubSP Salamae* was the most prevalent strain of *Salmonella spp.*, as shown in Table 6.

Table (6): *E. coli* and *salmonella* serotypes were isolated from different cattle slaughterhouses.

	Serotypes monovalent	Polyvalent	Percentage
E. coli serotypes	O44: K74	III	25%
	O142: K86	I	25 %
	O119: K69	II	25 %
	O164: K –	III	12.5 %
	O26: K60	I	12.5 %
Salmonella serotypes	*S. Enteric Sub SP Salamae*	-	100 %

4. DISCUSSION

To ensure the production of high-quality meat, slaughtering must take place in slaughterhouses supervised by veterinarians, with strict adherence to hygienic protocols (Elsharawy and Mahran, 2018). For the evaluation of slaughterhouse hygiene levels and the prevention of carcass contamination, microbiological data on hygiene indicators are crucial (Mrdovic et al., 2017). The operational facilities and hygienic protocols of various cattle slaughterhouses in Egypt were evaluated in this study.

The results showed that Slaughterhouse D obtained the highest biosecurity score, whereas Slaughterhouse B obtained the lowest score. This discrepancy can be attributed to the slaughterhouse (B) having fewer hygienic measures such as worker hygiene, foot baths, wheel dips, cleaning and disinfection programs, appropriateness of the location of the slaughterhouse, distance from the residential area and standard slaughterhouse design (Ahsan et al., 2020).

As an indicator of the quality of meat, TAPC is a valuable metric for determining the extent of bacterial contamination in food (Bogere and Baluka, 2014). Based on these findings, slaughterhouse (B) has the highest prevalence of TAPC, whereas slaughterhouse (C) demonstrated the lowest prevalence of TAPC. This can be attributed to the less-hygienic measures implemented in the slaughterhouse (B). The hand and floor samples contained the highest percentage of TAPC. This can be attributed to the employees neglecting hand hygiene and disinfection before and/or following slaughter (Ahsan et al., 2020). High TAPC on the floor because of an inadequate cleaning and disinfection program; also, bleeding and skinning are conducted on the floor in a horizontal position. While horizontal bleeding allows for

more rapid bleeding, it lacks the same level of hygiene as vertical bleeding (Mummed and Webb, 2015).

This study found that across the four slaughterhouses, the prevalence of *Staphylococcus spp*. was 88.33%. Similarly, Abunna et al. (2016) found that 53.2% of the samples tested positive for *Staphylococcus spp*. Our results showed that the prevalence of *Staphylococcus spp*. was the highest in Slaughterhouse B and the lowest in Slaughterhouse C. The lack of proper sanitation in the slaughterhouse is the main cause of *Staphylococcus spp.* contamination (Abunna et al., 2016). The meat and the atmosphere could be contaminated by the skin, mouth, sneezing, and spitting of the people inside the slaughterhouse (Morshdy et al., 2022). We found that *Staphylococcus spp.* was most abundant in floor swabs. The lack of a footbath, improper floor cleaning, disinfection, and the lack of visitors' hygiene could all contribute to this outcome (Ahsan et al., 2020).

Using PCR targeting the *NUC* gene, randomly selected *Staphylococcus spp.* were positively identified as *Staph. aureus* in this investigation. Also, the *BAP* gene is responsible for biofilm formation, yet only 60% of *Staph. aureus* isolates tested positive for PCR targeting this gene. These results were nearly similar to those of Munive Nuñez et al. (2023), who reported that 78.9% of the isolates carried the *BAP* gene. The ability of Staphylococcus aureus to form biofilm is considered to be a major virulence factor influencing its survival and persistence in both the environment and the host (Torlak et al., 2017).

Our results showed that the prevalence of *E. coli* across the four slaughterhouses was 39.58%. nearly similar to Bersisa et al. (2019), who revealed that the proportion of *E. coli* was 35.2%. According to our findings, slaughterhouse A had the maximum prevalence of *E. coli* and slaughterhouse D had the lowest prevalence. fecal contamination of the bovine epidermis related to *E. coli* contamination in beef (Abdissa et al., 2017). Our study indicated that the wall samples contained the highest prevalence of *E. coli*. This may be due to neglecting the cleaning and disinfection program of the wall from fecal contamination (Ahsan et al., 2020). According to the serological identification of *E. coli* isolates, the most popular *E. coli* serotypes in the cattle slaughterhouses were O44: K74, O142: K86, O119: K69, O164: K-, and O26: K60. In a prior investigation (Edris et al., 2012), O26:K60 and O119:K69 were also identified.

According to the results obtained from the current investigation, the prevalence of *Salmonella* across the four slaughterhouses was 2.29 percent. The findings of this study are nearly similar to those of Ketema *et al*. (2018), who reported a salmonella percentage of 3.7%. The low prevalence of *Salmonella* may be attributed to the washing of carcasses and various components of the slaughterhouses with water. According to Muluneh and Kibret (2015), *salmonella* prevalence at slaughterhouses can be significantly reduced through carcass washing. According to these findings, slaughterhouse C had the maximum prevalence of *Salmonella*, while slaughterhouse A was negative for *Salmonella*. *Salmonella* was most prevalent in the samples obtained from the skinning knife. This may have occurred because, during our investigation, we observed that only one knife was used during the skinning process without disinfecting it, and workers did not adequately clean their hands. It was determined that the microbial load on hands and knives could be substantially reduced by hand washing and dipping the blade of a knife into hot water at ≥82°C for 5 sec (Durmuşoğlu et al., 2020). *Salmonella* isolates were serologically identified, and *S. enterica sub sp. SP Salamae* was identified as the predominant serotype. Also, Cossi et al. (2014) identify *S. enterica Sub SP Salamae* but at low frequencies.

According to these results, water samples contain the lowest proportion of TAPC, *Staphylococcus spp., and E. coli* and are negative for *Salmonella spp*. The observed outcome may be attributed to the utilization of chlorinated water by all examined slaughter houses (Ellis-Iversen et al., 2009).

5. CONCLUSIONS

This study aimed to assess the effect of hygienic measures in slaughterhouses on the level of bacterial contamination. According to the findings of our study, there are several variables that contribute to meat contamination in slaughterhouses. These factors include inadequate hygienic measures, a lack of hygiene among personnel and incorrect meat handling. Therefore, it is very important to take appropriate management, good hygiene, and biosecurity measures in order to control the bacterial contamination of meat.

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