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RESEARCH ARTICLE

Evaluation of the Hygienic Status at El-Qurein Abattoir in Sharkia Governorate, Egypt

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Abstract

Cross-contamination of animal carcasses and their contact surfaces at any level of the meat handling process is a significant factor in the production of high-quality meat. The present study was carried out to investigate the hygienic status at El-Qurein abattoir, Sharkia Governorate, Egypt. Microbial indicators for the hygienic measures including total aerobic plate count (APC) and *Staphylococcus* count (TSC) were investigated. In addition, the prevalence of *Staphylococcus* species among abattoir samples and *Staphylococcus aureus* antimicrobial susceptibility testing were also detected. The obtained results revealed that the bacterial contamination in abattoir samples was as follows; abattoir effluents > floors > walls > brisket > rump with mean values of 5.89 ±0.01, 5.65±0.02, 5.06±0.01, 4.87±0.01, and 4.41±0.05 $\log_{10}$ CFU/cm^2, respectively for APC, while, 4.92 ± 0.02, 4.80 ± 0.02, 4.70 ± 0.02, 4.61 ± 0.03, and 4.38 ± 0.05 $\log_{10}$ CFU/cm^2 for TSC, respectively. The disc diffusion test of *S. aureus* isolates revealed its resistance to most of the tested antibiotics with high multiple antibiotic resistance (MAR) indices. It was concluded that the hygienic measures at El-Qurein abattoir were inadequate. This study suggested the necessity of the application of appropriate food safety practices inside the abattoir and the adoption of personal hygienic measures among abattoir workers.

Keywords: Abattoir samples, total aerobic count, *S. aureus*, antibiotic resistance

Introduction

Microbial contamination of animal carcass surfaces is primarily caused by the presence of a diverse range of germs in the meat slaughterhouse environment. Cross-contamination occurs at various stages of processing, including animal slaughter, flaying, evisceration, deboning, and carcass transportation, resulting in meat contamination with a wide variety of microorganisms, including food-poisoning organisms, with serious public health consequences [1,2]. Total aerobic plate count (APC) and total *Staphylococcus* count (TSC) are two microbial markers that provide a good overview of the sanitary conditions and measures taken during carcass handling and processing, and, as a result, have an impact on the production of high-quality meat [3]. *Staphylococcus aureus (S. aureus*) is one of the most prevalent foodborne pathogens, it can grow in a wide variety of temperatures, pH, and salt concentrations, as well as, it can adapt, survive, and colonize even in potentially dry and stressful environments, allowing it to thrive in slaughterhouses and meat [4]. *S. aureus* in food is a consequence of insufficient hygienic handling and processing, posing a possible risk to human health [5]. It generates heat stable and proteolytic enzyme-resistant enterotoxins that provoke food intoxication in humans, resulting in vomiting, abdominal discomfort, and diarrhea [6]. Antibiotic resistance in Staphylococci has a track record of developing rapidly and successfully. The

acquisition and transmission of antibiotic resistance plasmids and the possession of innate resistance mechanisms have resulted in this defensive reaction [7]. The implication of *S. aureus* as a persistent nosocomial and community-acquired pathogen has become a global health concern. It has a remarkable ability to evolve various resistance mechanisms to most antimicrobial drugs [8].

Thus, this study aimed to assess the hygienic status at El-Qurein abattoir, Sharkia Governorate as a potential for *S. aureus*. In addition, the aerobic plate count and the anti-microbial resistance profile of *S. aureus* isolates were investigated.

Material and methods

Sampling

Between March and August 2021, 100 samples were collected from two different sources: specific parts of beef carcasses and surfaces of the processing environment at El-Qurein city abattoir in Sharkia Governorate, Egypt. Using sterile swabs, the samples were collected from two different carcass regions (brisket and rump). Environmental samples were collected from the processing line's holding areas (abattoir floors, walls, and effluents). The collected swabs were suspended in 25 mL of 1% peptone water (Oxiod, CM9). Samples were kept in an ice tank and then immediately transferred to Food Control Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt for bacteriological analysis.

Bacteriological Examination

One mL of each 0.1% peptone water containing swabs was transferred into a sterile test tube containing 9 mL of 0.1% peptone water, then tenfold serial dilutions were prepared up to the required dilution [9]. The aerobic plate count was determined according to APHA [10]. The total APC = number of colonies x dilution factor. The count was presented as a colony-forming unit (CFU/cm^2). The plates with 30-300 colonies were counted. For *Staphylococcus* spp. count 0.1 mL from each prepared dilution was spread onto duplicate plates of Baird Parker agar (BP, Hi-Media, M043-500G, Mumbai India) supplemented with egg yolk tellurite emulsion (50 mL/L, Oxiod SR54) and incubated at 37ºC for 48 hours. Typical *Staphylococcus* colonies (circular, black, shiny colonies surrounded by a clear halo zone) were counted and recorded [10]. Gram staining and biochemical tests (catalase, coagulase, oxidase, and DNase) for identification of *S. aureus* were performed according to ISO 4833-1[11].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of 29 *S. aureus* isolates was carried out using Kirby-Bauer disk diffusion method against 16 antimicrobials according to Clinical Laboratory Standards Institute [12]. Antimicrobial discs were used: kanamycin (k, 30 µg), nalidixic acid (NA, 30 µg), cefotaxime (CF, 30 µg), tetracycline (T, 30 µg), clindamycin (CL, 10 µg), sulphamethoxazol (SXT, 25µg), ipipenem (IPM, 10µg), cefazolin (CZ, 30 µg), amoxycillin (AMX, 30 µg), meropenem (M, 10 µg), gentamicin (G, 10 µg), ciprofloxacin (CP, 5 µg), erythromycin (E, 15 µg), ampicillin (AM, 10 µg), amikacin (AK, 30 µg), oxacillin (OX, 1µg). Multiple antibiotic resistance (MAR) index was detected. MAR index = (a/b), where (a) is the number of antibiotics to which the isolates are resistant. (b): is the total number of the tested antibiotics [13].

Data analysis

All values of bacteriological analysis are presented as means ± standard error (S.E). Data were analyzed by SPSS (version XI) and One-Way Analysis of Variance (ANOVA) at a 95% level of confidence. Significant differences among the means were determined by the Duncan test considering $P < 0.05$ as significant. The results were expressed as the logarithm of the colony forming units per square centimeter (log_{10} CFU/cm^2).

Results

The results of the APC revealed that the examined abattoir effluent samples were the highest APC and ranged from 5.78 to 5.95 with a mean value of 5.89 ± 0.01 log_{10} CFU/cm^2 (Table 1). The APC of the examined carcasses surfaces samples showed that brisket samples had higher count of 4.87 ± 0.01 log_{10} CFU/cm^2 than rump samples (4.41± 0.05 log_{10} CFU/cm^2).

Table (1): Statistical analytical results of aerobic plate count in the examined abattoir samples

Swab samples*	Aerobic plate count (log_{10} CFU/cm^2)		
	Minimum	Maximum	Mean ± S. E
Brisket	4.78	4.95	4.87 ± 0.01^{d}
Rump	4.00	4.70	4.41 ± 0.05^{e}
Abattoir effluents	5.78	5.95	5.89 ± 0.01^{a}
Floors	5.48	5.78	5.65 ± 0.02^{b}
Walls	5.00	5.18	5.06 ± 0.01^{c}

*No. = 20 of each, S.E = Standard error of mean, CFU/cm^2: colony forming unit per square centimeter. Means were calculated on positive samples, means within the same column carrying different superscripts are significantly different at ($P < 0.05$) based on Duncan's multiple comparisons.

The results showed that *Staphylococcus* spp. count was the highest in abattoir effluent samples (4.92 ± 0.02 log_{10} CFU/cm^2) and in floor samples (4.80 ± 0.02 log_{10} CFU/cm^2). However, brisket and rump samples had the lowest count of 4.61± 0.03 and 4.38± 0.05 log_{10} CFU/cm^2, respectively (Table 2).

Table (2) Statistical analytical results of *Staphylococcus* spp. count in the examined abattoir samples

Swab samples*	*Staphylococcus* spp. count (log_{10} CFU/cm^2)		
	Minimum	Maximum	Mean ± S. E
Brisket	4.48	4.78	4.61 ± 0.03^{d}
Rump	4.00	4.70	4.38 ± 0.05^{e}
Abattoir effluents	4.78	5.00	4.92 ± 0.02^{a}
Floors	4.60	4.90	4.80 ± 0.02^{b}
Walls	4.60	4.78	4.70 ± 0.02^{c}

*No. = 20 of each. S.E = Standard error of mean, CFU/cm^2: colony forming unit per square centimeter. Means were calculated on positive samples, means within the same column carrying different superscripts are significantly different at ($P < 0.05$) based on Duncan's multiple comparisons.

Out of 100 swab samples, 29 *S. aureus* (29%) were isolated and identified (Table 3). Comparing the prevalence of *S. aureus* in the investigated swab samples, *S. aureus* was isolated from abattoir effluents, floor, and wall with higher overall percentages of 50% and 40%, and 30%, respectively than other surface samples (Table 3). The biochemical identification of *Staphylococcus* species showed that *S. aureus, S. epidermidis, S. xylosus,* and *S. intermedius* were detected in 29 (29%), 6 (6%), 3 (3%), and 2 (2%), respectively (Table 3).

Table (3) prevalence of *Staphylococcus* species in the examined abattoir samples (No. = 20 of each).

Samples	*S. aureus*	*S. epidermidis*	*S. xylosus*	*S. intermedius*
Brisket	3 (15%)	-	-	1 (5%)
Rump	2 (10%)	1 (5%)	-	-
Abattoir effluents	10 (50%)	3 (15%)	2 (10%)	-
Floors	8 (40%)	1 (5%)	1 (5%)	-
Walls	6 (30%)	1 (5%)	-	1 (5%)
Total	29 (29%)	6 (6%)	3 (3%)	2 (2%)

The results of the antibiotic susceptibility testing of 29 *S. aureus* isolates of abattoir swab samples showed that most of the isolates (93.1%) were sensitive to oxacillin, and 86.2% were sensitive to amikacin and ampicillin (Table 4). High resistance (100%) was detected to kanamycin and nalidixic, 89.7% to cefotaxime, and 72.4% to tetracycline (Table 4). Resistance profile of multidrug resistant *S. aureus* isolated from abattoir samples revealed that the MAR index ranged from 0.125 to 1 with an average of 0.54 (Table 5).

Table (4) Antimicrobial susceptibility testing of 29 *S. aureus* isolates from abattoir samples

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Kanamycin (K)	-	-	-	-	29	100
Nalidixic acid (NA)	-	-	-	-	29	100
Cefotaxime (CF)	2	6.9	1	3.4	26	89.7
Tetracycline (T)	5	17.2	3	10.4	21	72.4
Clindamycin (CL)	8	27.6	2	6.9	19	65.5
Sulphamethoxazol (SXT)	10	34.4	1	3.4	18	62.1
Ipipenem (IPM)	14	48.3	2	6.9	13	44.8
Cefazolin (CZ)	13	44.8	4	13.8	12	41.4
Amoxycillin (AMX)	17	58.6	3	10.4	9	31.0
Meropenem (M)	21	72.4	1	3.4	7	24.1
Gentamicin (G)	22	75.9	-	-	7	24.1
Ciprofloxacin (CP)	22	75.9	2	6.9	5	17.2
Erythromycin (E)	24	82.8	1	3.4	4	13.8
Ampicillin (AM)	25	86.2	-	-	4	13.8
Amikacin (AK)	25	86.2	1	3.4	3	10.4
Oxacillin (OX)	27	93.1	1	3.4	1	3.4

No.: Number of sensitive, intermediate or resistant *S. aureus* isolates. %: Percentage of sensitive, intermediate or resistant *S. aureus* isolates**.**

Table (5) Resistance profile of multidrug resistant 29 *S. aureus* isolates from abattoir samples

Pattern	Resistance profile	No.	No	MAR
I	K, NA, CF, T, CL, SXT, IPM, CZ, AMX, M, G, CP,	1	16	1
II	K, NA, CF, T, CL, SXT, IPM, CZ, AMX, M, G, CP,	2	15	0.938
III	K, NA, CF, T, CL, SXT, IPM, CZ, AMX, M, G, CP,	1	14	0.875
IV	K, NA, CF, T, CL, SXT, IPM, CZ, AMX, M, G, CP	1	12	0.750
V	K, NA, CF, T, CL, SXT, IPM, CZ, AMX, M, G	2	11	0.688
VI	K, NA, CF, T, CL, SXT, IPM, CZ, AMX	2	9	0.563
VII	K, NA, CF, T, CL, SXT, IPM, CZ	3	8	0.500
VIII	K, NA, CF, T, CL, SXT, IPM	1	7	0.438
IX	K, NA, CF, T, CL, SXT	5	6	0.375
X	K, NA, CF, T, CL	1	5	0.313
XI	K, NA, CF, T	2	4	0.250
XII	K, NA, CF	5	3	0.188
XIII	K, NA	3	2	0.125

No.: Number of isolates. No: Number of antibiotics. MAR: Multiple Antibiotic Resistance index (a/ b), where (a) is the number of antibiotics to which the isolates are resistant. (b): is the total number of the tested antibiotics (16). K: Kanamycin, NA: Nalidixic acid, CF: Cefotaxime, T: tetracycline, CL: Clindamycin, SXT: Sulphamethoxazol, IMP: Ipipenem, CZ: Cefazolin, AMX: Amoxycillin, M: Meropenem, G: Gentamicin, CP: Ciprofloxacin, E: Erythromycin, AM: Ampicillin, AK: Amikacin, OX: Oxacillin

Discussion

The aerobic plate count has been adopted as an indicator to assess the quality of abattoir and beef carcasses and, consequently, to predict the risk of meat consumption. The APC in brisket samples in the current study was higher than 2.54 ± 0.94 log_{10} CFU/cm^2 in Canada [14] and 3.1 ± 0.25 log_{10} CFU/cm^2 in Ireland [15]. High results of 6.9 log_{10} CFU/cm^2 were recorded in Texas, the United States, [16] and 5.10 ± 0.63 log_{10} CFU/cm^2 in New Zealand [17]. In rump samples, APC was nearly similar to 4.2 ± 2.2 log_{10} CFU/cm^2 in South Africa that reported by Pearce and Bolton [18]. Meanwhile, low count of 2.2 log_{10} CFU/cm^2 and 3.24 ± 0.02 log_{10} CFU/cm^2 were reported in Ireland [19] and in Sudan, respectively [20]. The higher contamination rate of brisket than rump may be attributed to multiple contacts with contaminated equipment and workers' hands especially at the evisceration stage. The carcasses being constantly suspended, undergo a shift of microbes from posterior to anterior, unlike the brisket, the rump has a significantly lower contamination rate due to its remote from the ground and workers' handling [21]. Regarding the abattoir effluent samples, the APC (log_{10} CFU/cm^2) was higher than 4.46 and 2.64 obtained by Ogunlade *et al*. [22] and Onuoha *et al*. [23], respectively in Nigeria. Higher count of 13.93 was found [24] in Nigeria. Higher viable counts noticed in abattoir effluent samples may be due to the wastes produced during abattoir practice with associated significant unhygienic ways of carcasses processing in the abattoir. The APC (log_{10} CFU/cm^2) for floor swab samples was nearly similar to 5.4 in Egypt [25] but exceeded 2.91 ± 1.23 in Turkey [26] and 4.2 in Nigeria [27]. Higher count of 6.59 ± 0.05 in Tanzania was reported by Ntanga et al. [28]. Floors are a significant source of contamination because they transmit contamination to workers' shoes, the workers, in turn, disperse within the abattoir, spreading the contamination. Nonetheless, abattoir effluents and floors can provide an encouraging environment for microbial activity, as well as, an essential source of spreading and maintenance of microbial cells, particularly if cleaned with high-pressure water. This practice has the potential to propagate contamination by suspending microorganisms in the air through water droplets [29]. APC of the examined wall samples was higher than 4.71 ± 1.2 log_{10} CFU/cm^2 that recorded in Algeria [30], 1 log_{10} CFU/cm^2 in Texas, United States [16], and 4.5 ± 0.09 in Egypt [31]. Higher results of 13.48 log_{10} CFU/cm^2 and 8.50 log_{10} CFU/cm^2 were reported in Nigeria [24] and in Egypt [29]**,** respectively. Higher bacterial count obtained from floor swab samples may be attributed to the presence of accumulated blood, animals' internal organs, and polluted water on the floor [25].

Contamination of meat by *Staphylococcus* spp. results from poor hygienic measures during slaughtering process. In the current study, *Staphylococcus* count (log_{10} CFU/cm^2) in brisket and rump samples was higher than 3.7 ± 2.2 and 3.9 ± 2.4 in South Africa [19], 2.37 and 2.98 in Egypt [32], respectively. Meanwhile, a high count of 12 ± 1.02 log_{10} CFU/cm^2 for brisket and 17 ± 1.1 log_{10} CFU/cm^2 for rump was detected in Egypt [33]. The total *Staphylococcus* count in abattoir effluents (4.92 ± 0.02 log_{10} CFU/cm^2) was similar to those found in India (4.87 log_{10} CFU/cm^2) [34] and lower than 16.5 log_{10} CFU/cm^2 recorded in Nigeria [35]. Regarding floor samples, the total *Staphylococcus* count in this study was nearly similar to 5.98 ± 0.07 log_{10} CFU/cm^2 in Ethiopia [36], higher than 3.85 ± 0.42 log_{10} CFU/cm^2 in India [37], and lower than 7.0 ± 5.0 log_{10} CFU/cm^2 in Nigeria [35] and 100 ± 18 log_{10} CFU/cm^2 in Egypt [38]. For wall swab samples, the total *Staphylococcus* count was lower than 18.7 and 5.4 log_{10} CFU/cm^2 in Egypt [29, 38], respectively. The variations of the results may be attributed to how the carcasses were handled and unsanitary practices observed during data collection [26].

The highest occurrence of *S. aureus* was 10 (50%) in abattoir effluents and the lowest value was 2 (10%) in rump samples. The lower contamination rate of rump samples might be attributed to the absence of enough

nutrients and oxygen needed by microorganisms to grow and multiply [39]. The occurrence of *S. aureus* in the brisket and rump samples was lower than 20% in South Africa [39]. *S. aureus* was isolated by percentage 33.3% and 73% from abattoir effluent samples in Nigeria [40,41], respectively. In addition, a higher percentage of 80% in Bangladesh was reported by Ahaduzzaman et al. [42]. *Staphylococcus* species in the abattoir effluents could develop from meat during slaughter practice, abattoir floors, beef processing, and the meat handlers. The skin, mouth, sneezing, and spitting activities of the people inside the abattoir could contaminate the meat and the environment with *S. aureus* [43]. *S. aureus* in floor samples in this study (40%) was higher than 25% in Nigeria [44] and 33.33% in Ethiopia [36]. The prevalence of *S. aureus* in wall samples (30%) was higher than 8% in Turkey [45] and 1.3% in Nigeria [46]. The differences in the results can be associated with lack of cleaning, improper handling, and contamination from polluted air [47].

Antimicrobial-resistant *S. aureus* has been related to uncontrolled usage of drugs as growth promoters and for treatment in food animals [48]. In accordance with the obtained results, *S.aureus* isolates have shown increased resistance to tetracyclines by percentages of 71.42% [49]. On contrary with our results, *S. aureus* resistance to amoxicillin (100%) showed by Ahaduzzaman *et al*. [42], in addition, Al-Hilua and Al-Shujairib [47] detected 100 % resistance to ampicillin, amoxicillin, and gentamicin. Furthermore, *S. aureus* resistance was 90% [50] and 100% [51] to oxacillin. Moreover, Iroha *et al.* [40] declared 100% resistance to oxacillin and 95% to erythromycin. Our results were in tandem with Tanih *et al*. [39] who found that *S. aureus* isolates were 100% resistant to nalidixic acid. The obtained results were in agreement with those obtained by Abd El Tawab *et al*. [52] who found *S. aureus* isolates were sensitive to gentamicin (90%) and ciprofloxacin (87.5%). Accordingly, the higher resistance for imipenem and meropenem in the current study than ampicillin, amoxycillin, and oxacillin may be due to imipenem acts as an antibiotic by preventing Gram-positive and Gram-negative bacteria from synthesizing their cell wall. It is a potent inhibitor of beta-lactamases from some Gram-negative bacteria that are resistant to most beta-lactam antibiotics, and it stays very stable in the presence of beta-lactamase generated by these bacteria. Different causes, however, are blamed for the establishment of resistance as time passes [53]. As a result, high resistance to carbapenems (imipenem and meropenem) could be owing to antibiotic inactivation caused by enzymatic action on the antibiotic's structure, as well as blocking access to a target by modifying the outer membrane permeability. The MAR index of 0.2 or more shows contamination from high-hazard sources, and so, posing dangers to consumers [54].

Conclusion

The results obtained from this study demonstrated the presence of *S. aureus* in carcasses and the processing environment at El-Qurein abattoir, Sharkia Governorate, Egypt. The high concentration of bacterial contaminants and multidrug resistance profile of *S. aureus* isolated from abattoir samples are an indication of the hygiene and safety of such abattoir. Therefore, suitable processing parameters and personal hygienic practice should be treated as important control measures to minimize and eliminate the hazard associated with these organisms. Moreover, it's necessary to control the usage of drugs as growth promoters and for treatment in food animals.

Conflict of Interest

None of the authors has any conflict of interest to declare.

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الملخص العربي

تقييم الوضع الصحي في مسلخ القرين بمحافظة الشرقية ، مصر

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يُعد تلوث ذبائح الحيوانات والأسطح الملامسة لها أثناء تداول اللحوم عاملاً هامًا في إنتاج اللحوم عالية الجودة. أجريت هذه الدراسة لمعرفة الحالة الصحية لمسلخ القرين بمحافظة الشرقية بجمهورية مصر العربية. تم فحص المؤشرات الميكروبية للقياسات الصحية بما في ذلك العدد الكلي للميكروبات الهوائية والعدد الكلي للمكورات العنقودية ، بالإضافة إلي ذلك ، تم الكشف عن إنتشار أنواع المكورات العنقودية بين العينات المجمعة وبخاصة المكورات العنقودية الذهبية وإختبار حساسيتها للمضادات الميكروبية. أظهرت النتائج أن التلوث البكتيرى في عينات المجزركان على النحوالتالي: مخلفات المجزر> الأرضيات الجدران مسحات الصدر مسحات الفخذ بمتوسط قيم (5.89 ± 0.01 و 5.65 ± 0.02 و 5.06 ± 0.01 و 4.87 ± 0.01 و 4.41 ± 0.05 لوغاريتم 10 لكل مستعمرة بكتيرية) علي التوالي بالنسبة للعدد الكلي للميكروبات الهوائية ، في حين كان متوسط القيم (4.92 ± 0.02 و 4.80 ± 0.02 و 4.70 ± 0.02 و 4.61 ± 0.03 و 4.38 ± 0.05 لوغاريتم 10 لكل مستعمرة بكتيرية) علي التوالي بالنسبة للعدد الكلي للمكورات العنقودية ،كما كشفت الدراسة مقاومة عزلات المكورات العنقودية الذهبية لمعظم المضادات الحيوية المختبرة بمعامل مقاومة عالي. خلصت الدراسة إلى عدم كفاية التدابير الصحية في مسلخ القرين. كما اقترحت هذه الدراسة ضرورة تطبيق ممارسات سلامة الغذاء المناسبة واعتماد تدابيرالنظافة الشخصية بين العاملين داخل المجزر.