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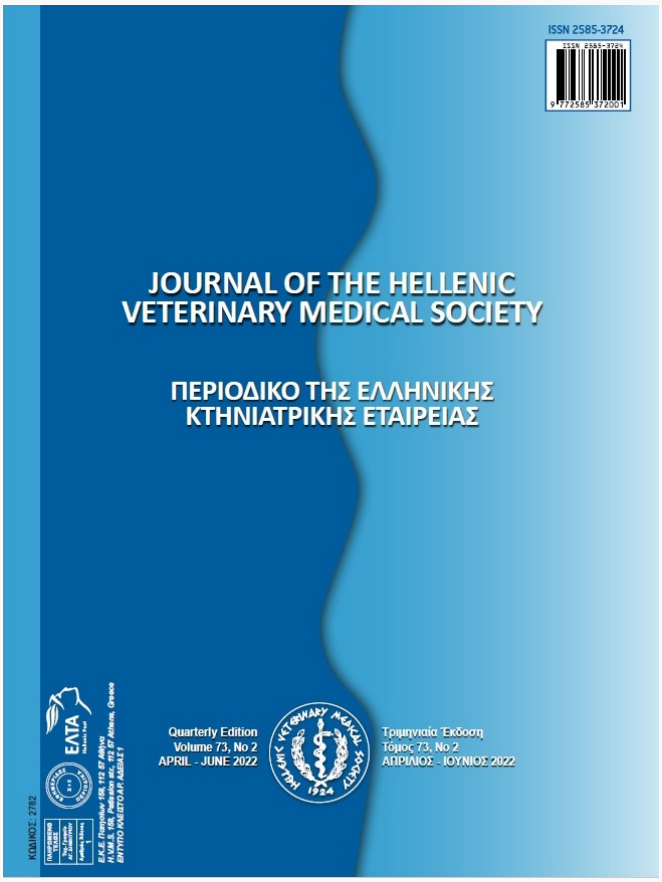

Prevalence and biofilm-formation ability of Staphylococcus aureus isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece

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Research article
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Prevalence and biofilm-formation ability of *Staphylococcus aureus* isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece

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ABSTRACT: *Staphylococcus aureus* is one of the leading causes of foodborne intoxications.The pathogen's biofilm-formation ability facilitates its spread and enhances its tolerance against hostile environments. The objectives of this cross-sectional study were to investigate the prevalence of *S. aureus* in the received livestock, the corresponding carcasses, the employees and the surfaces of infrastructures and tools in three abattoirs of Northern Greece and to determine the biofilm-forming potential of the recovered isolates. The isolation of presumptive *S. aureus* isolates from different types of samples was performed using classic microbiological methods and molecular identification to the species level was done via detection of the *coa* and *nuc* genes. Biofilm-formation ability was assessed using a semi-quantitative, microtiter plate method. Fifty-five out of 547 samples examined tested positive for the presence of *S. aureus*. The highest *S. aureus* isolation frequency was observed from human nasal cavities (17.2%) and tool surfaces (16.1%) followed by pig carcasses (15.5%), small ruminant nasal cavities (15.0%), cattle nasal cavities (7.5%), pig nasal cavities (6.9%), infrastructure surfaces (6.8%), cattle carcasses (5.7%) and small ruminant carcasses (5.0%). The isolation frequency of *S. aureus* varied considerably ($p < 0.05$) among the sampled establishments, ranging from 4.2% to 31.7%. All *S. aureus* isolates were found capable of producing biofilms: 43.6% possessed strong biofilm-formation ability, 54.5% moderate and only one isolate (1.8%) showed weak biofilm-formation ability. The contamination of equipment and tools by biofilm-producing *S. aureus* emphasizes the need for the application of strict hygiene practices during meat-processing. In addition, the application of an effective and regularly verified sanitation program is necessary to prevent biofilm formation and minimize the risk of carcass contamination.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen, colonizing the skin and the mucous membranes of humans and animals (Cuny *et al.*, 2013; Sollid *et al*., 2014).Notably, despite their host adaptation, some lineages of *S. aureus* spread from animals to humans and vice-versa (Peton and Le Loir, 2014). Its versatile nature, in terms of hosts and clinical manifestations, and its carriage of genes encoding various virulence traits (e.g., enterotoxigenicity, antimicrobial resistance, biofilm-formation ability), render *S. aureus* one of the most important pathogens (Lowy, 1998, 2003; Vanderhaeghen *et al.*, 2010; Peton and Le Loir, 2014). Hence, *S. aureus* is the causative agent of a variety of illnesses ranging from minor to severe skin infections, toxin-mediated diseases (i.e., food poisoning, scalded skin syndrome and toxic shock syndrome), and often life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis or bacteremia (Le Loir *et al.*, 2003; Tong *et al.*, 2015).From a food-safety viewpoint, *S. aureus* is one of the main causative agents of food-poisoning, with staphylococcal intoxication being the result of ingestion of foods containing preformed staphylococcal enterotoxins (Fox *et al.*, 2017).

The epidemiology of *S. aureus* in domestic animals, food of animal origin and food handlers along the food production chain is very important. Contamination of meat by *S. aureus* may happen during the slaughter of livestock, as well as during subsequent processing of meat and meat products (Mechesso *et al.*, 2021).In fact, different stages of the slaughtering process (i.e., evisceration, dressing), the on-line contact between different carcasses and the direct contact with contaminated tools and environmental surfaces are recognized as potential routes of transmission (Brusa *et al.*, 2019; Costa *et al.*, 2020).

In terms of public health, the ability of *S. aureus* to produce biofilms on biotic and abiotic surfaces is a critical attribute (Doulgeraki *et al.*, 2017). Biofilm formation is considered a growth mode of *S. aureus*, naturally encompassed within its environmental life-cycle (Miao *et al.*, 2017). In addition, biofilms not only support the adherence and colonization of *S. aureus* in nature (Costerton *et al.*, 1999), but also protect against the action of antimicrobial agents, host immune responses and the deleterious effects of cleaning agents (Lister *et al.*, 2009; Singh *et al.*, 2010). Notably, the enhanced biofilm-induced tolerance against common sanitation procedures that are applied at the food-processing environment, not only presents a considerable challenge to the successful eradication of *S. aureus* from the food processing equipment and other food-contact surfaces, but also increases the risk of cross-contamination and ultimately the risk of staphylococcal foodborne intoxication (Vázquez-Sánchez *et al.*, 2013; Lianou *et al.*, 2020).

To the best of our knowledge, literature data on the prevalence of biofilm-forming *S. aureus* in the meat production chain are limited. Therefore, the aim of the present study was to: (i) investigate the prevalence of *S. aureus* in the animals (cattle, pigs, small ruminants) destined to be slaughtered, in the resulting carcasses and in the employees and the surfaces of the infrastructures and tools of three abattoirs located in Northern Greece, and (ii) determine the biofilm-formation ability of the *S. aureus* isolates.

MATERIALS AND METHODS

Sampling

A total of 547 samples were retrieved from three randomly selected abattoirs (B, K and L) of variable maximum production capacity, throughout different administrative districts of Northern Greece. Abattoir B is classified as of "high production capacity", whereas abattoirs L and K are classified as of "medium production capacity" according to the relevant National legislation (Joint Ministerial Council Decision 2014/1221-50912, 2014). The collected samples originated from the carcasses (n=184) and the nasal cavities (n=184) of 184 animals [cattle (n=106), pigs (n=58), small ruminants (n=20)], the nasal cavities of abattoir workers (n=58) and 121 were environmental samples [infrastructure surfaces (n=59), tool surfaces (n=62)].

The sampling of animals' nasal cavities was conducted right after stunning, by using the same swab-stick for both nasal cavities. A pooled carcass swab sample was collected from each animal immediately after postmortem inspection, including four sampling areas of 100 cm^2 each, according to the relevant National legislation (Joint Ministerial Council Decision 2014/1545-70158, 2014) guidelines on sampling of carcasses at the abattoir level. In addition, a bi-lateral nasal (anterior nares) swab was taken from all workers (who participated voluntarily). Surface sampling was performed by swabbing a minimum area of 100 cm^2 (or the maximum available area, in case of smaller tools) using swab-sticks moistened in buffered pep-

tone water (BPW, LAB M, Lancashire, United Kingdom).

All samples were collected aseptically using sterile swabs along with single-use, screw-capped tubes filled with Stuart transport medium (Stuart Sterile Swab; Deltalab, Barcelona, Spain). All samples were transported to the laboratory under refrigerated conditions in less than 4 hours from the time of sampling.

Isolation and identification of *Staphylococcus aureus*

Upon arrival to the laboratory, each sample was immediately transferred to a test tube filled with 10ml of Tryptone Soy broth (TSB; LAB M) supplemented with 6.5% (w/v) NaCl (Merck, Darmstadt, Germany) and 0.3% (w/v) yeast extract (YE, LAB M). After an 18-hour incubation at 37 ºC, 10μl of the pre-enriched broth was surface-plated onto Baird-Parker Agar (BPA; LAB M) supplemented with egg-yolk tellurite (EYT, LAB M) and the plates were aerobically incubated at 37 ºC for 48 hours. Up to four presumptive *S. aureus* colonies (black colonies surrounded by an opaque zone and a zone of clearing around the opaque zone) from each plate were sub-cultured on Tryptone Soya Agar (TSA; LAB M) for 24 hours at 37 ºC and then were subjected to Gram staining, along with mannitol fermentation testing and catalase-testing (O'Brien *et al.*, 2012). Furthermore, all suspect colonies were subjected to a rapid test (Microgen Staph Rapid Test; Microgen Bioproducts, Surrey, UK) for the detection of the coagulase enzyme and the protein A, assisting the tentative identification to the species level (*S. aureus*). Among them, one presumptive *S. aureus* isolate per sample was randomly chosen and stored under freezing conditions (-80 °C) in cryotubes containing TSB with 20% glycerol for further investigation.

Molecular characterization of *Staphylococcus aureus*

All phenotypically presumptive-positive *S. aureus* isolates were submitted to PCR tests targeting the *coa* and the species-specific *nuc* genes. Genomic DNA was extracted using the Pure Link Genomic DNA kit (PL DNA kit; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentration was determined spectrophotometrically using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Massachusetts, USA).The PCR conditions used were previously described by Zdragas *et al.* (2015)and the relevant primer sets for the detection of the *coa* and *nuc* genes were those described by Hookey *et al.* (1998) and Sudagidan and Aydin (2009), respectively. The amplified DNA products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and were visualized under UV illumination (TEX-20 M, Life Technologies, Gibco BRL System).

Biofilm-formation ability

A semi-quantitative, microtiter-plate (MTP), adherence assay, originally described by Wang *et al.* (2010), was used to assess the ability of *S. aureus* strains to produce biofilms *in vitro*. In brief, *S. aureus* isolates were cultured overnight at 37 ºC in TSB supplemented with 0.25% glucose and then diluted to 10^8 CFU/ml using the same (sterile) medium. Two hundred μl of each culture was transferred to individual wells of a 96-well, polystyrene, microtiter plate (COSTAR 3596, Cole-Parmer, Illinois, USA) and incubated aerobically at 37 ºC for 24 hours. Afterwards, each well was carefully washed three times by using 200 μl of sterile 0.9% NaCl to remove loosely attached cells. Samples were then stained by adding 100 μl of a 0.3% (w/v) crystal violet solution. Five minutes later, the excess staining was removed by rinsing gently with water three times. Following de-staining with ethanol, the microtiter plate was air-dried and the optical density (OD) of adherent biofilms was spectrophotometrically measured at 570 nm.

The cutoff optical density (ODc) was defined as the mean OD value of the negative control (plain broth medium). Depending on the resulting OD measurement, *S. aureus* strains were characterized according to Borges *et al.* (2012) as no biofilm producers (OD ≤ODc), weak biofilm producers (ODc< OD ≤ 2 × ODc), moderate biofilm producers (2 × ODc< OD ≤ 4 × ODC), or strong biofilm producers (4 × ODc< OD).

Statistical Analysis

Statistical analyses were performed using XLSTAT (v. 2021.1.1.1090, Addinsoft, New York, USA). Contingency tables were used to provide the frequency distribution of the presence of *S. aureus* and biofilm-formation ability, per abattoir and sample type. Chi-square tests were used for the comparisons of proportions. Statistical significance was assessed using an alpha of 0.05.

RESULTS AND DISCUSSION

Prevalence of *Staphylococcus aureus*

All presumptive *S. aureus* isolates were found to carry the *coa* and *nuc* genes, confirming their identification to the species level. Hence, the pathogen was isolated from 55 out of the 547 tested samples yielding an overall detection frequency of 10.1%, comparable to that previously reported (11.7%) in a similar study performed in an abattoir of Northern Greece (Drougka *et al.*, 2019). The number of samples collected per establishment, along with the corresponding isolation frequencies of *S. aureus* per tested sample type are presented in Table 1.

The isolation frequency of *S. aureus* varied considerably ($p< 0.05$) among the three sampled establishments (Table 1). The highest isolation frequency (31.7%) was noted in abattoir L, followed by abattoir K (14.2%).The lowest isolation frequency was observed in abattoir B (4.2%), which is the only establishment of high production capacity (industrial abattoir) operating in the context of certified standard procedures, as dictated by the established Food Safety Management System (FSMS). With respect to the prevalence of *S. aureus*, considerable differences were observed even among the two medium-scale capacity abattoirs (K and L), which nevertheless differ in size and the potential production volume. Hence, the application of a more systematic approach in terms of the slaughtering process and the assisting procedures together with a greater availability of resources (including training), both of which are usually associated with bigger establishments (such as abattoir B), could have contributed to the smaller overall frequency of detection of *S. aureus* in abattoir B. However, such comparisons should be done with caution because of the relatively low overall number of samples tested, the lack of testing of samples from small ruminants and workers from abattoir L and of cattle samples from abattoir K.

S. aureus was detected in all types of collected samples (Table 1). The overall isolation frequency of *S. aureus* from animal nasal and carcass samples was similar (8.2% and 8.7%, respectively). At the animal species level, the highest isolation frequency of *S. aureus* was noted in pig carcasses (15.5%), followed by samples from small ruminant nasal cavities (15.0%), cattle nasal cavities (7.5%), pig nasal cavities (6.9%), cattle carcasses (5.7%) and small ruminant carcasses (5.0%). However, the high overall prevalence of *S. aureus* in pig carcasses and nasal cavities was due to their recovery from abattoir L (21.4% and 7.1% from carcass and nasal cavity samples, respectively) and abattoir K (19.4% and 9.7% from carcass and nasal cavity samples, respectively); no *S. aureus* was recovered from samples collected from pig nasal cavities and the corresponding carcasses from abattoir B.

The findings from previously published relevant studies are quite variable. Drougka *et al.* (2019) reported a lower (8.0%) isolation frequency of *S. aureus* from pig carcasses of a Greek abattoir, and a zero prevalence in samples from pig nasal cavities.

Table 1. Prevalence of *S. aureus* in samples collected from three abattoirs of Northern Greece

Sample type	Abattoir B		Abattoir L		Abattoir K		Total	
	n	*S. aureus* (%)	n	*S. aureus* (%)	n	*S. aureus* (%)	n	*S. aureus* (%)
Cattle	184	1 (0.5)	28	13 (46.4)	nt	-	212	14 (6.6)
Nasal cavities	92	1 (1.1)	14	7 (50.0)	nt	-	106	8 (7.5)
Carcass	92	0 (0)	14	6 (42.9)	nt	-	106	6 (5.7)
Pigs	26	0 (0)	28	4 (14.3)	62	9 (14.5)	116	13 (11.2)
Nasal cavities	13	0 (0)	14	1 (7.1)	31	3 (9.7)	58	4 (6.9)
Carcass	13	0 (0)	14	3 (21.4)	31	6 (19.4)	58	9 (15.5)
Small ruminants	10	1 (10.0)	nt	-	30	3 (10.0)	40	4 (10.0)
Nasal cavities	5	1 (20.0)	nt	-	15	2 (13.3)	20	3 (15.0)
Carcass	5	0 (0)	nt	-	15	1 (6.7)	20	1 (5.0)
Environment	78	6 (7.7)	7	3 (42.9)	36	5 (13.9)	121	14 (11.6)
Infrastructure surfaces	34	1 (2.9)	1	0 (0)	24	3 (12.5)	59	4 (6.8)
Tool surfaces	44	5 (11.4)	6	3 (50.0)	12	2 (16.7)	62	10 (16.1)
Human nasal cavities	38	6 (15.8)	nt	-	20	4 (20.0)	58	10 (17.2)
Animals' nasal cavities	110	2 (1.8)	28	8 (28.6)	46	5 (10.9)	184	15 (8.2)
Animals' carcasses	110	0 (0)	28	9 (32.1)	46	7 (15.2)	184	16 (8.7)
Total samples	**336**	**14 (4.2)**	**63**	**20 (31.7)**	**148**	**21 (14.2)**	**547**	**55 (10.1)**

nt, not tested

The isolation frequency of *S. aureus* from pig nasal cavities was higher (15.2%) in a study conducted in the Czech Republic (Klimešová *et al.*, 2017), while in another study conducted in Greece a higher isolation frequency (37.2%) of *S. aureus* was noted in tonsil samples of slaughtered pigs (Pexara *et al.*, 2020). O'Sullivan *et al.* (2011) reported a3.5% isolation frequency of *S. aureus* from the tonsils of pig carcasses in Canada. A higher isolation frequency (33.7%) was reported in pig carcasses at Ethiopia (Tefera *et al.*, 2019), whereas a much lower isolation frequency (2.8%) was reported at Nigeria for the same specimen type (Okorie-Kanu *et al.*, 2020). Although relatively limited data are available on the prevalence of *S. aureus* in healthy cows' nasal cavities, our estimates are higher (7.5%) than those reported in other recent studies from Greece (0.0%;Drougka *et al.*, 2019) and Spain (1.4%;Mama *et al.*, 2019). Similarly, our data indicate a slightly higher (5.7%) isolation frequency from cattle carcasses than that (4.5%) reported by Drougka *et al.* (2019). Of note, comparable results were reported from Sergelidis *et al.* (2015) (7.0%), but a higher estimate (12.2%) was reported from Drougka *et al.* (2019) regarding the *S. aureus* isolation frequency from small ruminant carcass samples at slaughter in Greece. Similarly, an 8.0% overall isolation frequency of *S. aureus* from healthy goat carcasses was reported in Korea (Mechesso *et al.*, 2021). However, considerably higher frequencies of *S. aureus* nasal carriage in small ruminants were reported from Saudi Arabia (41.0%), Denmark (43.4%) and the Czech Republic (31.7%) (Alzohairy, 2011; Eriksson *et al.*, 2013; Klimešová *et al.*, 2017), as well as from two different studies in Tunisia (44.8% in sheep, 19.2% in goats) (Gharsa *et al.*, 2012, 2015). The variation in the prevalence estimates reported in the aforementioned studies could be attributed to variations in the sensitivity of the detection methods used (Furuya *et al.*, 2007), along with variations concerning the experimental designs and the origin of tested samples.

In the present study the overall isolation frequency of *S. aureus* from human nasal swabs was17.2%, with no significant differences ($p = 0.687$) between the two abattoirs (Table 1). The corresponding prevalence reported from another Greek abattoir (Drougka *et al.*, 2019) was only slightly higher (20.8%). However, a carriage around 20% (persistent carriers) is considered typical for the general healthy population (Kluytmans *et al.*, 1997). Two related studies in Nigeria reported lower frequencies (13.5% and 6.7%) of *S. aureus* nasal carriage among abattoir workers (Odetokun *et al.*, 2018; Okorie-Kanu *et al.*, 2020).

Fourteen of the 121 environmental samples (11.6%) were positive for *S. aureus*, but differences were observed in the isolation frequency across the establishments. The highest isolation frequency was noted in samples from abattoir L (42.9%), followed by abattoir K (13.9%) and abattoir B (7.7%). The overall isolation frequency of *S. aureus* was 16.1% (10/62) from tool surfaces and 6.8% (4/59) from infrastructure surfaces. The higher prevalence of *S. aureus* on portable tools (as opposed to surfaces) maybe attributed to their inadequate sanitation during their sequential use on different carcasses. Furthermore, consistent with our findings, Beyene *et al.* (2017) reported a higher (33.3%) *S. aureus* occurrence in environmental specimens originating from knives and slaughter hanging equipment. With respect to the overall *S. aureus* occurrence in the abattoir environment, a higher (18.3%) estimate was reported from the study of Drougka *et al.* (2019) and a much higher (30.0%) estimate was reported from an abattoir in Ethiopia (Tefera *et al.*, 2019); however, a lower (3.3%) estimate was reported from investigations in two abattoirs in Nigeria (Odetokun *et al.*, 2018). The diversity in the findings from the different establishments of the same study, as well as in the findings of different studies could be associated with establishment-specific differences in terms of adopted hygiene practices which could lead to inadequate sanitation of specific environmental surfaces. Besides, substandard hygiene practices are frequently observed at the abattoir level (Beyene *et al.*, 2017), highlighting the importance of personnel training with respect to adherence to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). The insufficient implementation of GHP during slaughter may be reflected by the high isolation frequency of *S. aureus* from the animal carcasses. Such a connection is implied by the results of our study, particularly with respect to the prevalence of *S. aureus* in the pig carcasses of the two smaller abattoirs.

Biofilm-formation ability of *Staphylococcus aureus* isolates

Biofilm-formation ability is an important virulence trait of bacteria, protecting them from a variety of biocides and antibiotics, promoting horizontal exchange of antibiotic-resistance determinants and increasing the risk of cross contamination in food producing facilities (Savage et al., 2013; Angelidis et al., 2020). In our study 43.6% of the *S. aureus* isolates

Table 2. Biofilm-formation ability of *S. aureus* isolated from three Greek abattoirs according to sample type

Sample type	N	Biofilm-formation ability (%)		
		Weak	Moderate	Strong
Cattle nasal cavities	8	1 (12.5)	5 (62.5)	2 (25.0)
Cattle carcasses	6	0 (0)	2 (33.3)	4 (66.7)
Pig nasal cavities	4	0 (0)	2 (50.0)	2 (50.0)
Pig carcasses	9	0 (0)	3 (33.3)	6 (66.7)
Small ruminant nasal cavities	3	0 (0)	2 (66.7)	1 (33.3)
Small ruminant carcasses	1	0 (0)	0 (0)	1 (100.0)
Infrastructure surfaces	4	0 (0)	4 (100.0)	0 (0)
Tool surfaces	10	0 (0)	5 (50.0)	5 (50.0)
Human nasal cavities	10	0 (0)	7 (70.0)	3 (30.0)
Total	**55**	**1 (1.8)**	**30 (54.5)**	**24 (43.6)**

N, number of isolates

possessed strong, 54.5% moderate, and only one isolate (1.8%) showed weak biofilm-formation ability. The distribution of the *S. aureus* isolates with respect to their biofilm-formation ability across the different sample types and abattoirs is presented in Table2. A non-significant ($p = 0.113$) difference was noted in the isolates' biofilm-formation ability among abattoirs. In fact, within the different abattoirs, the greatest difference in biofilm-formation ability was observed in the 14 isolates of abattoir B (11 moderate, 3 strong and no isolates with weak biofilm-formation ability). The 21 isolates from abattoir K were more homogeneously distributed among the moderate and strong classes in terms of biofilm-formation ability (12 moderate *vs.*9 strong), but like the situation in abattoir B, no isolates with weak biofilm formation ability were recovered. Strong biofilm-producers dominated the pool of 20 isolates from abattoir L (12 strong, 7 moderate, 1 weak). Isolates characterized as strong biofilm-producers were more frequently isolated from animal carcasses (11/55, 20.0%) than from animal nasal cavities (5/55, 9.1%) but no significant difference was found ($p > 0.05$).

Limited data are available in the literature regarding the biofilm formation ability of *S. aureus* occupational isolates; nonetheless, our study revealed that 30% of the human *S. aureus* isolates possessed strong biofilm-formation ability and the remaining isolates possessed a moderate biofilm-formation ability (Table 2).

The isolation frequency of *S. aureus* from the environmental samples of all abattoirs was11.6% (Table 1); this could pose a significant food contamination hazard (Gibson *et al.,* 1999). Among the 14 environmental isolates, five (35.7%) were characterized as strong producers and nine (64.3%) were classified as moderate producers (Table 2). All isolates recovered from infrastructure surfaces were characterized as moderate producers, whereas isolates recovered from tools were equally divided between moderate (recovered from abattoir B) and strong producers (recovered from abattoirs L and K).The overall high occurrence of biofilm-forming *S. aureus* in the environmental samples could indicate a consideration point with regards to the applied cleaning methods, starting from the design of sanitation protocols through their effective verification. Papadopoulos *et al.* (2019) made a similar suggestion regarding an equally challenging environment (dairy industries).

Literature data on the biofilm-formation ability of food-related *S. aureus* isolates recovered from the food production chain are limited (Di Ciccio *et al.*, 2015).Ou *et al.* (2020)reported that *S. aureus* isolates with biofilm-formation ability was commonly recovered from foods of animal origin in Shanghai andmost of the isolates were deemed strong producers (64.8%) compared to moderate (20.0%) and weak producers (15.2%). However, similar to our findings, isolates lacking biofilm-formation ability were not recovered at all. In addition, Di Ciccio *et al.* (2015) observed a high prevalence of *S. aureus* strains with biofilm-formation ability in food contact surfaces (50.0%) and food handlers (22.7%); both these estimates are higher than the corresponding estimates in the present study (11.6% and 17.2%, respectively).

CONCLUSIONS

The detection of *S. aureus* capable of biofilm formation in the examined livestock, their corresponding carcasses, the food handlers and especially the processing environment of the abattoirs is of great

importance. The data suggest that the abattoirs may act as an introduction point for the pathogen into the meat supply chain. The ability to form biofilms not only facilitates the dissemination of *S. aureus,* but also enhances its tolerance against the applied sanitation methods, while at the same time it could promote the horizontal exchange of antibiotic-resistance determinants. Therefore, a thorough implementation of the Good Animal Welfare Practices, the GMP and the GHP is warranted. These implementations need to start at the farm level and consider the presence of *S. aureus* strains with biofilm-formation ability during the design of sanitation strategies, which should be strictly implemented and regularly verified. However, a more extensive epidemiological surveillance of *S. aureus* throughout the meat-chain is needed to better understand the pathogen's dynamics for dissemination in this food sector.

CONFLICT OF INTEREST

None declared by the authors.

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