



Journal of the Hellenic Veterinary Medical Society

Vol 73, No 2 (2022)



To cite this article:

Komodromos, D., Kotzamanidis, C., Giantzi, V., Angelidis, A. S., Zdragas, A., & Sergelidis, D. (2022). Prevalence and biofilm-formation ability of Staphylococcus aureus isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece. *Journal of the Hellenic Veterinary Medical Society*, *73*(2), 4097–4104. https://doi.org/10.12681/jhvms.26469

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

D. Komodromos^a, C. Kotzamanidis^b, V. Giantzi^b, A.S. Angelidis^c, A. Zdragas^b, D. Sergelidis^a

^a Laboratory of Hygiene of Foods of Animal Origin - Veterinary Public Health, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

^bHellenic Agricultural Organization - DEMETER, Veterinary Research Institute of Thessaloniki, Campus of Thermi, Thermi, Greece

^cLaboratory of Safety and Quality of Milk and Dairy Products, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

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.

Corresponding Author: Sergelidis Daniel,Laboratory of Hygiene of Foods of Animal Origin - Veterinary Public Health, School of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece E-mail address: dsergkel@vet.auth.gr

Date of initial submission: 21-03-2021 Date of acceptance: 30-03-2021

INTRODUCTION

taphylococcus aureus (S. aureus) is an opportu-Distic 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 lifecycle (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 swabstick for both nasal cavities. A pooled carcass swab sample was collected from each animal immediately after postmortem inspection, including four sampling areas of 100 cm² 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 cm² (or the maximum available area, in case of smaller tools) using swab-sticks moistened in buffered peptone 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 au*reus

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 °C, 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 °C 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 °C 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 °C in TSB supplemented with 0.25% glucose and then diluted to 108 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 (CO-STAR 3596, Cole-Parmer, Illinois, USA) and incubated aerobically at 37 °C 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 $100 \,\mu$ l 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 \leq ODc), weak biofilm producers (ODc< OD \leq 2 × ODc), moderate biofilm producers (2 × ODc< OD \leq 4 × ODC), or strong biofilm producers (4 × ODc< OD).

Statistical Analysis

Statistical analyses were performed using XL-STAT (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 (Droug-ka *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

Table 1 Dravalance of C. guyoug in complex collected from three shotteins of Northe

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. revalence of 5. aureus in samples conceled nom unce abattons of Normeni Orecce									
	Aba	ittoir B	Ab	attoir L	Ab	attoir K	Total		
Sample type	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 (Ode-

tokun 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

Samula trina	N	Biofilm-formation ability (%)					
Sample type	IN	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.

REFERENCES

- Alzohairy MA (2011) Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia. *J Bacteriol Res* 3: 63-68.
- Angelidis AS, Komodromos D, Giannakou R, Arsenos G, Gelasakis AI, Kyritsi M, Filioussis G, Hadjichristodoulou C, Torounidou P, Papa A, Sergelidis D (2020) Isolation and characterization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) from milk of dairy goats under low-input farm management in Greece. *Vet Microbiol* 247: 108749.
- Beyene T, Hayishe H, Gizaw F, Beyi AF, Abunna F, Mammo B, Ayana D, Waktole H, Abdi RD (2017) Prevalence and antimicrobial resistance profile of *Staphylococcus* in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. *BMC Res Notes* 10: 1-9.
- Borges S, Silva J, Teixeira P (2012) Survival and biofilm formation by Group B streptococci in simulated vaginal fluid at different pHs. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol* 101: 677-682.
- Brusa V, Restovich V, Signorini M, Pugin D, Galli L, Díaz VR, Arias R, Leotta GA (2019) Evaluation of intervention measures at different stages of the production chain in Argentinian exporting abattoirs. *Food Sci Technol Int* 25: 491-496.
- Costa M, Pracca G, Sucari A, Galli L, Ibargoyen J, Gentiluomo J, Brusa V, Martinez Zugazua M, Figueroa Y, Londero A, Roge A, Silva H, Van Der Ploeg C, Signorini M, Oteiza JM, LeottaGA (2020) Comprehensive evaluation and implementation of improvement actions in bovine abattoirs to reduce pathogens exposure. Prev Vet Med 176: 104933.
- Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: A common cause of persistent infections. *Science* 5418: 1318-1322.
- Cuny C, Köck R, Witte W (2013) Livestock associated MRSA (LA-MR-SA) and its relevance for humans in Germany. Int J Med Microbiol 303: 331-337.
- Di Ciccio P, Vergara A, Festino AR, Paludi D, Zanardi E, Ghidini S, Ianieri A (2015) Biofilm formation by *Staphylococcus aureus* on food contact surfaces: Relationship with temperature and cell surface hydrophobicity. *Food Control* 50: 930-936.
- Doulgeraki AI, Di Ciccio P, Ianieri A, Nychas GJE (2017) Methicillin-resistant food-related *Staphylococcus aureus*: a review of current knowledge and biofilm formation for future studies and applications. *Res Microbiol* 168: 1-15.
- Drougka E, Foka A, Giormezis N, Sergelidis D, Militsopoulou M, Jelastopulu E, Komodromos D, Sarrou S, Anastassiou ED, Petinaki E, Spiliopoulou I (2019) Multidrug-resistant enterotoxigenic *Staphylococcus aureus* lineages isolated from animals, their carcasses, the personnel, and the environment of an abattoir in Greece. *J Food Process Preserv* 43: 1-10.
- Eriksson J, Espinosa-Gongora C, Stamphøj I, Larsen AR, Guardabassi L (2013) Carriage frequency, diversity and methicillin resistance of *Staphylococcus aureus* in Danish small ruminants. *Vet Microbiol* 163: 110-115.
- Fox A, Pichon B, Wilkinson H, Doumith M, Hill RLR, McLauchlin J, Kearns AM (2017) Detection and molecular characterization of Livestock-Associated MRSA in raw meat on retail sale in North West En-

gland. Lett Appl Microbiol 64: 239-245.

- Furuya EY, Cook HA, Lee MH, Miller M, Larson E, Hyman S, Della-Latta P, Mendonca EA, Lowy FD (2007) Community-associated methicillin-resistant *Staphylococcus aureus* prevalence: How common is it? A methodological comparison of prevalence ascertainment. *Am J Infect Control* 35: 359-366.
- Gharsa H, Ben Slama K, Lozano C, Gómez-Sanz E, Klibi N, Ben Sallem R, Gómez P, Zarazaga M, Boudabous A, Torres C (2012) Prevalence, antibiotic resistance, virulence traits and genetic lineages of *Staphylococcus aureus* in healthy sheep in Tunisia. *Vet Microbiol* 156: 367-373.
- Gharsa H, Slama K Ben, Gómez-Sanz E, Lozano C, Zarazaga M, Messadi L, Boudabous A, Torres C (2015) Molecular characterization of *Staphylococcus aureus* from nasal samples of healthy farm animals and pets in Tunisia. *Vector-Borne Zoonotic Dis* 15: 109-115.
- Gibson H, Taylor JH, Hall KE, Holah JT (1999) Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *J Appl Microbiol* 87: 41-48.
- Hookey J V., Richardson JF, Cookson BD (1998) Molecular typing of Staphylococcus aureusbased on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J Clin Microbiol 36: 1083-1089.
- Joint Ministerial Council Decision 2014/1221-50912 concerning the calculation of production capacity of the ungulates' slaughterhouses (2014). http://www.et.gr/idocs-nph/search/pdfViewerForm. html?args=5C7QrtC22wEc63YDhn5AeXdtvSoClrL8fnjEuKFUx-ZjNZ8op6Z_wSuJInJ48_97uHrMts-zFzeyCiBSQOpYnTy36MacmUFCx2ppFvBej56Mmc8Qdb8ZfRJqZnsIAdk8Lv_e6czmhEemb-NmZCMxLMtQQE9Xr5UrKWle8AtM9La-vmJjo4Ag9Bv8PEYun-FZYV6 [accessed on 10.03.2021]
- Joint Ministerial Council Decision 2014/1545-70158 concerning the procedures' setting for sampling and laboratory analysis of products of animal origin, during the official inspection at the slaughterhouses of ungulates animals and poultry (2014). http://www.et.gr/idocs-nph/ search/pdfViewerForm.html?args=5C7QrtC22wEc63YDhn5AeXdtvSoClrL8VngElbqsA6B_zJjLAILKFuJInJ48_97uHrMts-zFzey-CiBSQOpYnTy36MacmUFCx2ppFvBej56Mmc8Qdb8ZfRJqZnsI-Adk8Lv_e6czmhEembNmZCMxLMtfqqRsjsYEHdLq6cidKqV-3V9ShVYXxcL2NQ85KIoRyG1 [accessed on 10.03.2021]
- Klimešová M, Manga I, Nejeschlebová L, Horáček J, Ponížil A, Vondrušková E (2017) Occurrence of *Staphylococcus aureus* in cattle, sheep, goat, and pig rearing in the Czech Republic. *Acta Vet Brno* 86: 3-10.
- Kluytmans J, Van Belkum A, Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 10: 505-520.
- Le Loir Y, Baron F., Gautier M (2003) Staphylococcus aureus and food poisoning. Genetics and Molecular Research. Genet Mol Res 2: 63-76.
- Lianou A, Nychas GJE, Koutsoumanis KP (2020) Strain variability in biofilm formation: A food safety and quality perspective. *Food Res*

Int 137: 109424.

- Lister JL, Horswill AR (2014) Staphylococcus aureus biofilms: Recent developments in biofilm dispersal. Front Cell Infect Microbiol 4: 1-9.
- Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant *Pseudo-monas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* 22: 582-610.
- Lowy FD (1998) Staphylococcus aureusinfections. N Engl J Med 339: 520-532.
- Lowy FD (2003) Antimicrobial resistance: The example of Staphylococcus aureus. J Clin Invest 111: 1265-1273.
- Mama OM, Gómez-Sanz E, Ruiz-Ripa L, Gómez P, Torres C (2019) Diversity of staphylococcal species in food producing animals in Spain, with detection of PVL-positive MRSA ST8 (USA300). *Vet Microbiol* 233: 5-10.
- Mechesso AF, Moon DC, Ryoo GS, Song HJ, Chung HY, Kim SU, Choi JH, Kim SJ, Kang HY, Na SH, Yoon, Soon S, Lim SK (2021) Resistance profiling and molecular characterization of *Staphylococcus au*reus isolated from goats in Korea. *Int J Food Microbiol* 336: 108901.
- Miao J, Liang Y, Chen L, Wang W, Wang J, Li B, Li L, Chen D, Xu Z (2017) Formation and development of *Staphylococcus* biofilm: With focus on food safety. *J Food Saf* 37: 1-11.
- O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, Forshey BM, Kulick ME, Wallinga DB, Smith TC (2012) MRSA in conventional and alternative retail pork products. *PLoS One* 7: 3-8.
- O'Sullivan T, Friendship R, Blackwell T, Pearl D, McEwen B, Carman S, Slavićc D, Dewey C (2011) Microbiological identification and analysis of swine tonsils collected from carcasses at slaughter. *Can J Vet Res* 75: 106-111.
- Odetokun IA, Ballhausen B, Adetunji VO, Ghali-Mohammed I, Adelowo MT, Adetunji SA, Fetsch A (2018) *Staphylococcus aureus* in two municipal abattoirs in Nigeria: Risk perception, spread and public health implications. *Vet Microbiol* 216: 52-59.
- Okorie-Kanu OJ, Anyanwu MU, Ezenduka E V., Mgbeahuruike AC, Thapaliya D, Gerbig G, Ugwuijem EE, Okorie-Kanu CO, Agbowo P, Olorunleke S, Nwanta JA, Chah KF, Smith TC (2020) Molecular epidemiology, genetic diversity and antimicrobial resistance of *Staphylococcus aureus* isolated from chicken and pig carcasses, and carcass handlers. *PLoS One* 15: 1-18.
- Ou C, Shang D, Yang J, Chen B, Chang J, Jin F, Shi C (2020) Prevalence of multidrug-resistant *Staphylococcus aureus* isolate swith strong biofilm formation ability amonganimal-based food in Shanghai. Food Control 112: 107106

Peton V, Le Loir Y (2014) Staphylococcus aureus in veterinary medicine.

Infect Genet Evol 21: 602-615.

- Pexara A, Solomakos N, Govaris A (2020) Occurrence, antibiotic resistance and enteroxigenicity of *Staphylococcus* spp. in tonsils of slaughtered pigs in Greece. *Lett Appl Microbiol* 71: 394-399.
- Savage VJ, Chopra I, O'Neill AJ (2013) Staphylococcus aureus biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother 57: 1968-1970.
- Sergelidis D, Papadopoulos T, Komodromos D, Sergelidou E, Lazou T, Papagianni M, Zdragas A, Papa A (2015) Isolation of methicillin-resistant *Staphylococcus aureus* from small ruminants and their meat at slaughter and retail level in Greece. *Lett Appl Microbiol* 61: 498-503.
- Singh R, Ray P, Das A, Sharma M (2010) Penetration of antibiotics through *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *J Antimicrob Chemother* 65: 1955-1958.
- Sollid JUE, Furberg AS, Hanssen AM, Johannessen M (2014) Staphylococcus aureus: Determinants of human carriage. Infect Gen Evol 21: 531-541.
- Sudagidan M, Aydin A (2009) Screening virulence properties of staphylococci isolated from meat and meat products. *Wien Tierarztl Monatsschr* 96: 128-134.
- Tefera M, Aleme H, Girma S, Ali A, Gugsa G, Abera F, Muzeyin R, Legesse T, Gobena W, Fentaw S, Abubaker R, Tadesse A, Gonfa A (2019) Antimicrobial susceptibility pattern of *S. aureus* isolated from sheep and goat carcasses. *Open Microbiol J* 13: 16-20.
- Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG (2015) Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 28: 603-661.
- Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P (2010) Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiol Infect* 138: 606-625.
- Vázquez-Sánchez D, Habimana O, Holck A (2013) Impact of food-related environmental factors on the adherence and biofilm formation of natural *Staphylococcus aureus* isolates. *Curr Microbiol*. 66:110-121.
- Wang L, Yu F, Yang L, Li Q, Zhang X, Zeng Y (2010) Prevalence of virulence genes and biofilm formation among *Staphylococcus aureus* clinical isolates associated with lower respiratory infection. *African J Microbiol Res* 4: 2566-2569.
- Zdragas A, Papadopoulos T, Mitsopoulos I, Samouris G, Vafeas G, Boukouvala E, Ekateriniadou L, Mazaraki K, Alexopoulos A, Lagka V (2015) Prevalence, genetic diversity, and antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated from bulk tank milk from Greek traditional ovine farms. *Small Rumin Res* 125: 120-126.