Research Article

Enumeration and Antibiotic Resistance of *S. aureus* Isolated from Different Meat in Souk Ahras Area, Algeria

Sofiane Tamendjari[1,2*], Farida Bouzebda Afri[1,2], Lina Chaib[1], Hebib Aggad[3], Zoubir Bouzebda[1,2]

[1]Department of Veterinary Science, Institute of Agronomic and Veterinary Sciences, Mohammed Cherif Messaadia University, Souk Ahras, Algeria; [2]Laboratory of Animal Productions, Biotechnologies and Health (PABIOS), Institute of Agronomic and Veterinary Scien, Algeria; [3] Laboratory of Hygiene and Animal Pathology, Ibn Khaldoun University, Tiaret, Algeria. Algeria.

Abstract | *Staphylococcus aureus* is one of the main causes of infections in humans and animals, and it is a contaminant of food and can produce toxins where a real danger of food poisoning for the consumer. In addition, *S. aureus* are becoming increasingly resistant to antibiotics, leading to therapeutic failures. It is in this context that our study aims to have an idea on the degree of hygiene of meats produced and consumed in the wilaya of Souk Ahras with respect to these strains, and to evaluate their degrees of resistance to certain antibiotics used in human and veterinary medicine A total of 90 samples of sheep, chicken and turkey meat were collected and analyzed. Modified Baird-Parker medium was used for enumeration and isolation of strains, free coagulase and thermonuclease tests were performed as well as other biochemical tests. Antibiotic susceptibility testing was performed to evaluate the resistance of *S. aureus* to antibiotics. Finally, statistical processing was performed using the STATISTICA 7 software (Statsoft, France). The percentage of samples contaminated with *S. aureus* was 32.22%, and the average load of *S. aureus* contaminating sheep, chicken and turkey meat was 3.8×10^3 CFU/cm^2 +/- 2.55×10^3; 2.2×10^3 CFU/g +/- 0.99×10^3 and 2.01×103 CFU/g +/- 0.73×10^3 respectively. High rates of resistance to penicillin (100%), sulfonamides (93.10%) and multiple resistances were reported in this study. Staphylococci, coagulase-positive Staphylococci and in particular *S. aureus* are hygiene indicators that should not be neglected. For this reason, the general provisions of the slaughterhouse must be respected, the hygiene conditions improved and the professionals of the sector trained for good production and manufacturing practices. In addition, the use of antibiotics in a reasoned manner and the monitoring of these strains are strongly recommended.

Keywords | Sheep meat, Chicken meat, Turkey meat, Enumeration, Coagulation test, *S. aureus*, Antibiotic resistance, Algeria.

Received | February 11, 2022; **Accepted** | August 20, 2022; **Published** | January 15, 2023
***Correspondence** | Sofiane Tamendjari, Department of Veterinary Science, Institute of Agronomic and Veterinary Sciences, Mohammed Cherif Messaadia University, Souk Ahras, Algeria. Laboratory of Animal Productions, Biotechnologies and Health (PABIOS), Institute of Agronomic and Veterinary Scien, Algeria; **Email:** sofianetam06@gmail.com
Citation | Tamendjari S, Afri Bouzebda F, Chaib L, Aggad H, Bouzebda Z (2023). Enumeration and antibiotic resistance of *S. aureus* isolated from different meat in souk ahras area, Algeria. Adv. Anim. Vet. Sci. 11(2): 211-218.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.2.211.218
ISSN (Online) | 2307-8316

INTRODUCTION

Food safety is an important aspect of public health, fundamental to good health and sustainable development (WHO, 2021).

The 2018 World Bank report on the economic burden of foodborne disease estimates that annual production losses due to foodborne disease in low and middle-income countries are 95.2 US $ billion, with annual treatment costs of 15 US $ billion (WHO, 2020).

Foodborne illnesses are usually infectious or toxic and are caused by bacteria, viruses, parasites or chemicals that enter the body through contaminated food or drink. Despite significant progress made in many countries regarding food safety, millions develop illnesses each year as a result of eating contaminated food.

The pathogenic bacteria that cause a significant number of foodborne illnesses each year worldwide are *Salmonella enterica*, *E.coli*, *Campylobacter jejuni*, *Staphylococcus aureus* and *Listeria monocytogenes* (Sarhane et al., 2014).

S. aureus is involved in numerous foodborne illness outbreaks related to a wide variety of foods in many countries (Grace and Fetsch, 2018). It is responsible for approximately 292,000 cases per year in the Netherlands (Mangen et al., 2015), 241,000 cases in the United States (Scallan et al., 2011), 25,000 cases in Canada (Thomas et al., 2013), and 1300 cases in Australia (Kirk et al., 2014).

In Algeria, the Ministry of Commerce has recorded 3160 cases of collective food poisoning during the first half of 2021, without giving details of the agents responsible, note to mention the many people who prefer self-medication for fear of COVID-19 at the hospital level. Most studies done on *S. aureus* in foods, especially milk and dairy products, indicate that it is present at different levels and mainly informs us about its resistance to antibiotics. The resistance of *S. aureus* to antibiotics is another major problem that should be monitored.

Previous work on *S. aureus* resistance has revealed that *S. aureus* can act as a donor and recipient of resistance genes, many resistance genes are located on mobile genetic elements. The acquisition of new resistance genes by *S. aureus* is ongoing, resulting from its interaction with other bacteria (Kadlec et al., 2012; Wendlandt et al., 2013).

These strains are responsible for treatment failures and thus limit treatment options for severe infections, leading to increased costs of prevention and medical care (Cuny et al., 2013).

The Algerian network on antimicrobial resistance is in an alarming situation with regard to multidrug-resistant strains including *S. aureus*. According to the January 20, 2022 report by Philippa Roxby in BBC Africa, more than 1.2 million people died worldwide in 2019 from infections caused by antibiotic-resistant bacteria, more than the annual number of deaths caused by malaria or HIV. Antibiotic-resistant *S. aureus* is particularly deadly, as are other resistant bacteria. These data reveal a need for monitoring of antibiotic resistant strains and appropriate and judicious use of antimicrobials. The objective of our study is to evaluate the sanitary quality of meat consumed in the wilaya of Souk Ahras with respect to *S. aureus* and their resistance to some of the most commonly used antibiotics in human and veterinary medicine.

MATERIALS AND METHODS

Area and period of study

The study took place at the laboratory of Animal Productions, Biotechnology and Health, at the Institute of Agronomic and Veterinary Sciences, University of Souk Ahras, from March 2018 to September 2020.

Sampling

We collected 90 meat samples, namely 30 sheep meat samples, 30 chicken meat samples and 30 turkey meat samples (Table 1).

Table 1: region, location, origin and number of samples

Region	location	Origine	Number of samples
Souk Ahras	Slaughterhouse	Ovine carcass	30
Souk Ahras	Butchery	Chicken meat	15
Souk Ahras	Butchery	Turkey meat	15
Taoura	Butchery	Chicken meat	15
Taoura	Butchery	Turkey meat	15

The ovine meat samples were taken from ovine carcasses after stamping and before drying, according to the destructive method (ISO 17604 : 2003) with the help of a scalpel, and put in sterile bottles, at the communal slaughterhouse of Souk Ahras.

The chicken and turkey meat samples were taken from the breast at 12 different butcher shops in the commune of Souk Ahras and Taoura, wilaya of Souk Ahras, Algeria.

The samples are put directly into sterile bottles, transported to the laboratory in a cooler and analyzed the same day.

Sample processing

For sheep samples, a volume of 100 ml of previously sterilized peptone water is added to the samples from each sheep carcass to obtain a 10^{-1} stock solution, and for chicken and turkey meat samples, a volume of nine times the sample weight is added to obtain a 10^{-1} stock solution after homogenization (ISO 6887-1 : 2017).

Isolation and enumeration of *S. aureus*

For the isolation of *S. aureus*, Baird-Parker culture medium (Conda pronadisa, Spain) was used in which egg yolk and potassium tellurite (Giolliti Cantoni Additive, Institut Pasteur, Algeria) were added for better selection (ISO

6888-3 : 2003).

Enumeration was performed after 48 hours of incubation at 37 °C for *S. aureus* presumptive Staphylococci showing black, domed colonies, a clear aureole after 24 hours of incubation, and surrounded by an opaque halo after 48 hours of incubation for 10^{-2} and 10^{-3} dilutions, with the following formula : $N = \Sigma C / V \times 1.1 \times d$ (ISO 7218 : 2007).

N : number of microorganisms present in the sample.
ΣC : sum of colonies counted from the two dilutions retained namely 10^{-2} and 10^{-3}, one of the two boxes contains at least 10 colonies.
V : volume inoculated, i.e. 1 ml.
d : the dilution rate of the first dilution retained for the counts, i.e. 10^{-2}.

Presumptive *S. aureus* colonies are transferred to the heart-brain broth for better enrichment and purification on Mannitol Salt Agar (Chapman) for further testing.

For confirmation, Gram staining, Mannitol degradation, catalase, free coagulase, and thermostable DNase on DNA medium were performed.

Antibiotic susceptibility

All *S. aureus* strains were tested against some of the most used antibiotics in human and veterinary medicine by Muller-Hinton agar diffusion method in order to create a sensitivity and resistance profile (Ammari, 2011; CLSI, 2015).

The antibiotic discs (Liofilchem, Roeseto, Italy) used and their concentrations are: Penicillin (P) (10), Cefoxitin (FOX) (30), Gentamycin (CN) (10), Ofloxacin (OFX) (5), Erythromycin (E), Lincomycin (MY) (15), Tetracycline (TE) (30), Fosfomycin (FOS) (50), Sulfonamide-Trimethoprim (SXT) (1. 25/23.75), Sulfonamide (SMZ) (50), Fusidic acid (FC) (10), Chloramphenicol (30).

Statistical analysis

The comparison of means and degree of closeness between the isolated strains were performed using STATISTICA 7 software (Statsoft, France).

RESULTS

Prevalence of Staphylococcus

The number of samples contaminated with Staphylococci showing black, round, bulging, shiny colonies with a clear and/or opaque halo after 48 hours of incubation (Figure 1) is 63.33% (19/30) for sheep and turkey meat, and 53.33% (16/30) for chicken meat.

Figure 1: Presomptive colonies of *S. aureus*

Prevalence of coagulase positive Staphylococcus

The number of samples contaminated with coagulase positive Staphylococci for sheep meat is 46.66% (14/30), for chicken meat 23.33% (7/30) and 36.66% (11/30) for turkey meat.

Prevalence of *S. aureus*

The number of samples contaminated with *S. aureus* for sheep meat is 40% (12/30), for chicken meat is 23.33% (7/30), and for turkey meat is 33.33% (10/30) (Figure 2).

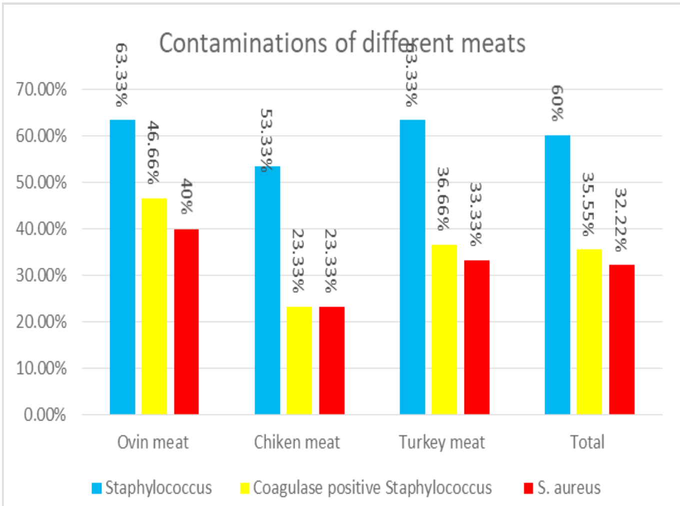

Figure 2: Contaminations of different meat samples

Enumeration of Staphylococcus

The average Staphylococcus load for sheep meat is 3.63×10^3 CFU/cm² while the higher and lower values are 11.5×10^3 CFU/cm² and 1.75×10^3 CFU/cm² respectively.

The average Staphylococcus load for chicken meat is 1.9×10^3 CFU/gr while the higher and lower values are 3.65×10^3 CFU/gr and 8×10^2 CFU/gr respectively.

The average Staphylococcus load for turkey meat is 1.9×10^3 CFU/gr while the higher and lower values are 3.15×10^3

Links Researchers

Table 2: Frequency of resistance and susceptibility of isolated *S. aureus* to the antibiotics tested

Antibiotics	Ovin (12)	Chiken (7)	Turkey (10)	Total (29)
Pénicilline[a]	12 (100%)	7 (100%)	10 100%	29 (100%)
Cefoxitin[g]	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gentamycin[f]	3 (25%)	2 (28.57%)	0 (0%)	5 (17.24%)
Ofloxacin[g]	0 (0%)	1 (14.28%)	1 (10%)	2 (6.89%)
Erythromycin[g]	2 (16.66 %)	0 (0 %)	0 (0%)	2 (6.89%)
Lincomycin[g]	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tetracyclin[d]	2 (16.66%)	2 (28.57%)	3 (30%)	7 (24.13%)
Fosfomycin[e]	0 (0%)	0 (0%)	1 (10%)	1 (3.45%)
Sulfonamide/Trimethoprim[f]	1 (8.33 %)	2 (28.57%)	2 (20%)	5 (17.24%)
Sulfonamide[b]	10 (83.33%)	7 (100%)	10 (100%)	27 (93.10%)
Fusidic acid[c]	3 (25%)	3 (42.85%)	3 (30%)	9 (31.03%)
Chloramphenicol[g]	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[a,b,c,d,e,f,g,] significant difference

CFU/gr and 1.1×10^3 CFU/cm2 respectively.

Enumeration of coagulase positive Staphylococcus

The average of coagulase positive Staphylococcus load for sheep meat is 3.9×10^3 CFU/cm^2 while the higher and lower values are 11.5×10^3 CFU/cm^2 and 1.75×10^3 CFU/cm^2 respectively.

The average of coagulase positive Staphylococcus load for chicken meat is 2.2×10^3 CFU/gr while the higher and lower values are 3.65×10^3 CFU/gr and 8×10^2 CFU/gr respectively.

The average of coagulase positive Staphylococcus load for turkey meat is 1.9×10^3 CFU/gr while the higher and lower values are 3.15×10^3 CFU/gr and 1.1×10^3 CFU/gr respectively.

Enumeration of *S. aureus*

The average *S. aureus* load for sheep meat is 3.8×10^3 CFU/cm^2 +/- 2.55×10^3 CFU/cm^2 while the higher and lower values are 11.5×10^3 CFU/cm^2 and 1.75×10^3 CFU/cm^2 respectively.

The average *S. aureus* load for chicken meat is 2.2×10^3 CFU/gr +/- 0.99×10^3 CFU/gr while the higher and lower values are 3.65×10^3 CFU/gr and 8×10^2 CFU/gr respectively.

The average *S. aureus* load for turkey meat is 2.01×10^3 CFU/gr +/- 0.73×10^3 CFU/gr while the higher and lower values are 3.15×10^3 CFU/gr and 1.1×10^3 CFU/gr respectively.

Frequences of isolated *S. aureus* susceptibility

The results of the susceptibility test (Figure 3 and Figure 4) are presented in Table (2).

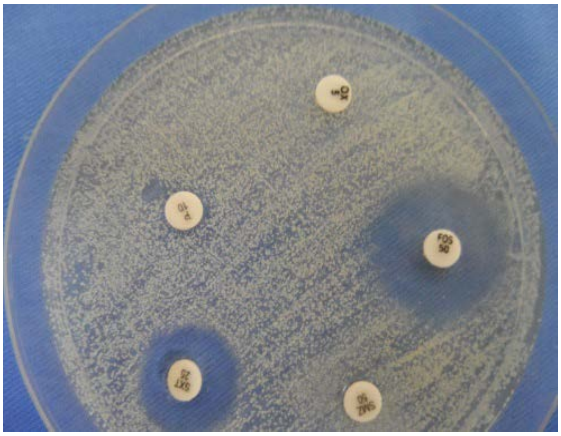

Figure 3: Results of antibiogram

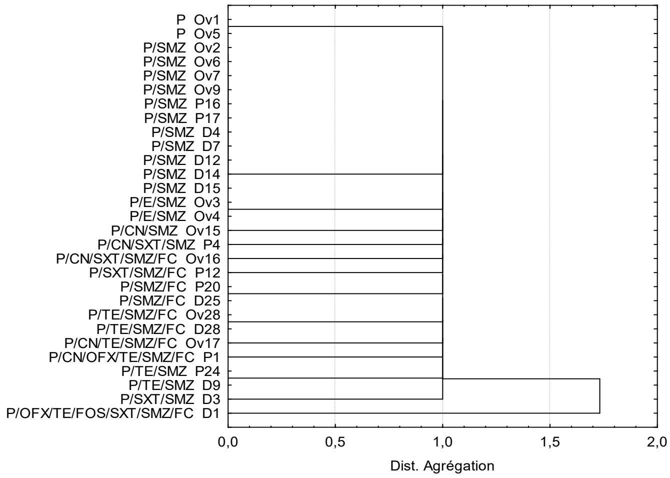

Figure 4: *S. aureus* isolated from different meats according to their antibiotic resistance profiles

DISCUSSION

The contamination rate of our samples by *S. aureus* is 35.55%, 40% for sheep meat, 23.33% for chicken meat and 33.33% for turkey meat with a significant difference ($p < 0.05$).

Our results are close to the results reported in Algeria by other authors who worked on chicken meat 46.66% (Guergueb et al., 2014), on raw meat and merguez 29.46% (Achek, 2018) and on raw meat 29.4% (Chaalal et al., 2018), or in Ghana (34%) for ready-to-eat meat (Adzitey et al., 2020), in Korea (33.2%) (Kim et al., 2020) and Cambodia (38.2%) (Rortana et al., 2021) for chicken meat and in Turkey (21. 23%) (Şanlıbaba, 2022) for red raw meats, however low prevalences are reported by Bouzid et al. (2015) (8.3%) and Titouche et al. (2020) (7.05%) having worked on minced meat in Algeria, 14.4% in Italy by Basanisi et al. (2020) for retail meats, and 10.58% in Iran for sheep meats (Baghbaderani et al., 2020). In Bangladesh, a high prevalence (54.9%) is reported for frozen chicken meat (Parvin et al., 2021).

Muscle is sterile, for it to become meat, it would need to be worked on and would need time. Both of these are favorable for processing and maturation, but also favorable for contamination, especially as the meat is handled. These contaminations are inevitable during slaughter, during evisceration by the hands of the workers, by the equipment, by the water used and possibly by the contents of the digestive tank.

The average contamination load by coagulase-positive Staphylococci is 3.9×10^3 CFU/cm^2, or 3.6 log10 CFU/g, for sheep carcasses, and 100% of the sampled carcasses exceeded the contamination load tolerated by Algerian regulations, which is [10^2-10^3] CFU/g (JORADP, 2017).

The average contamination load of coagulase-positive Staphylococci for chicken and turkey meat is 2.2×10^3 CFU/g (3.34 log10 CFU/g) and 1.9×10^3 CFU/g (3.28 log10 CFU/g) respectively, 100% of the samples are within Algerian standards (JORADP, 2017). But according to the European regulation which is 5000 CFU/g for poultry parts (Afssa, 2006) and 1000 CFU/g for poultry and poultry cuts since 2020 (FCD, 2019), our samples are not in the standards.

Our results are higher than those reported by Djenidi (2016) and Hamoudi et al. (2013), whose average load of Staphylococci and coagulase-positive Staphylococci on ovine and bovine carcasses are 2.22 log10 CFU/cm^2 in Sétif, and 2.15 log10 CFU/cm2 in Tiaret, respectively, in Algeria.

Our results of *S. aureus* contaminated chicken are lower than those reported by Akermi et al. (2020) (4.09 log10 CFU/g) in western Algeria, but higher than those reported by Guerguab et al. (2014) (1.08 log10 CFU/g) in Algeria, and by Sarhane et al. (2014) (2.67 log10 CFU/g) in Morocco.

The average load of coagulase-positive Staphylococci contaminating turkey meat was 3.28 log10 CFU/g, which is higher than that reported by Hamiroune et al. (2017) (2.02 log10 CFU/g) in Algiers, Algeria.

Some authors who have worked on red meats have reported bacterial loads higher than our results whether for Staphylococci, coagulase positive Staphylococci or *S. aureus* (Gebeyehu et al., 2013; Hachemi et al., 2019; Teshome et al., 2020), while others have reported lower loads (Hamiroune et al., 2017; Dib et al., 2019; Boukili et al., 2019).

These differences in bacterial loads from one country to another, or from one region to another, depend on the methods used at the level of processing workshops among others slaughterhouses. These methods include compliance with general provisions and training of staff for compliance with safety practices that could affect direct or indirect contamination and increase the bacterial load.

Although these contaminations are unavoidable, it is possible to reduce them. It was noticed in the slaughterhouse that the carcasses are worked on the ground with no separation of the sectors (clean / soiled), no respect of the forward walk, and the cutters do not have specific clothes. At the butcher's level, it was observed that the butchers do not wear gloves, no smock or smocks stained with blood, no cap, use of the same equipment and wooden surfaces such as tree trunks for cutting and preparing meat, a surface that allows bacteria to encrust, moreover even if the cold is respected, it does not sanitize the food. These conditions increase the likelihood of contamination compared to the regulations of the developed countries, besides some studies have reported that the coagulase positive Staphylococci begin to cause foodborne illnesses and produce free toxins in the food from 10^5 CFU/g of food (Ciupescu et al., 2018).

The highest instance of resistance (Table 4) is observed for Penicillin (100%) and Sulfonamides (93.10%) followed by Fusidic acid (31.03%), Tetracycline (24.14%), Sulfonamides-trimethoprim (17.24%) and Gentamycin (17.24%). Our results agree with those of Achek et al. (2018) and Titouche et al. (2020) on chicken meat, minced meat and other foods, reporting high rates of resistance to Penicillin. This may be due to the misuse of this antibiotic in these regions. Lower prevalence is reported by other authors observing different meats in Algeria (Challal et al., 2018; Hachemi et al., 2019), Turkey (Şanlıbaba, 2022), Bangladesh (Parvin et al., 2021), Iran (Baghbaderani et al., 2020) and Korea (Kim et al., 2020).

A high rate of resistance is also worrying for sulfonamides (93.10%), as this antibiotic is widely used in poultry farming. For fusidic acid, sulfonamides-Trimethoprim and gentamycin, lower rates are also recorded by some authors in Algeria or in the world (Achek et al., 2018; Hachemi et al., 2019; Islam et al., 2019; Titouche et al., 2020; Kim et al., 2020; Parvin et al., 2021). These antibiotics are mostly used in human medicine, but other authors have reported higher rates (Baghbaderani et al., 2020; Şanlıbaba, 2022). Lower resistances are observed for fosfomycin, Ofloxacin, and Erythromycin, and no resistance was observed for Cefoxitin, lincomycin, and Chloramphenicol. Some of these antibiotics are not used in veterinary medicine, and others are little known by the community for self-medication while others are banned in Algeria like Chloramphenicol. But other countries have recorded higher prevalences (Kazemi et al., 2019; Kim et al., 2020; Şanlıbaba, 2022).

It should be noted that there are 14 different resistance phenotypes, and according to the CLSI (2008) definition, 55.17% have multiple resistance, 37.93% have dual resistance, and 6.89% have single resistance to the antibiotics tested.

In several studies, a large proportion of *S. aureus* strains isolated from meats were also found to be multi-resistant (Abdalrahman et al., 2015; Normanno et al., 2015; Fox et al., 2017; Li et al., 2017). Livestock could be an important ecological niche for the emergence of multidrug-resistant *S. aureus*, as the extensive use of antibiotics for treatment, disease prevention, or growth promotion provides the necessary evolutionary constraints (Yan et al., 2014). With respect to community contamination and infection, a multidrug-resistant pathogen is an emerging concern for all meat types (Petternel et al., 2014).

CONCLUSION AND RECOMMANDATIONS

This study shows the importance of contamination of sheep carcasses at the communal slaughterhouse, chicken and turkey meat at some butcher shops by Staphylococcus, coagulase positive Staphylococcus and *S. aureus*.

These bacteria of human and/or animal origin are an indicator of hygiene and a pathogen not to be neglected since the contamination is done during the stages of transformation either by the animal or by the professionals of the trade. This work provides valuable information on the hygienic quality of these meats which are an important cause of food poisoning and collective food poisoning and it is essential to educate and train professionals in the meat industry to respect good hygiene practices in this region to reduce contamination by pathogenic bacteria.

Moreover, isolated strains are resistant to several antibiotics, and high prevalence of resistance is concerning for penicillin and sulfonamides, hence the need for a rational use of antibiotics and the monitoring of the evolution of these pathogenic and antibiotic resistant strains.

ACKNOWLEDGEMENTS

The authors thankfully the slaughterhouse staff and butchers for their kind cooperation in sample collection. The authors did not receive any funds for this study.

CONFLICT OF INTEREST

None of the authors have any conflit of interest to declare.

AUTHORS CONTRIBUTION

ST and LC : Sample collection, microbiological analysis of samples. ST : Supply of some products. Writing of the manuscript and statistical analysis. FAB, HA, ZB : Follow-up of the study, supply of some products, and correction of the manuscript.

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