African Journal of Microbiology Research

Full Length Research Paper

Contamination of meat products by coagulase positive staphylococci in the Algiers, Algeria

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The meat is regarded as one of the main sources of food-borne diseases; its evaluation can constitute a valuable source of information that can be used in the design of the collective prophylaxis programs in public health. In order to assess the level of contamination of certain meat products by coagulase-positive staphylococci and the influence of the storage temperature of these products, we conducted a study in three types of trade in the region of Algiers (Algeria). In total, 25 samples divided between meat products from red meat (minced meat, Merguez) and white meat (turkey, chicken) were taken at three different sales outlets (covered market, supermarkets and independent butchers), for bacteriological analysis. The mean of coagulase positive staphylococci were 1.60 ± 0.33 $\log_{10}$ cfu/g for minced meat, 2.02 ± 0.46 $\log_{10}$ cfu/g for Merguez, 2.02 ± 0.47 $\log_{10}$ cfu/g for turkey and 1.63 ± 0.21 $\log_{10}$ cfu/g for chicken. In addition, the descriptive analysis of the storage temperature data for these meat products revealed that, these temperatures have low correlations with variations in bacterial levels for minced meat, Merguez and turkey (minced). These results reflected insufficient hygienic conditions in the preparation, preservation and sale of these meat products. Thus, the consumption of these products can present a real health risk to public health.

Key words: Algeria, coagulase positive staphylococci, contamination, meat products, storage temperature.

INTRODUCTION

Meat is considered as a food of choice because of its nutritional value. Its richness in protein and the nature of these make it an indispensable food for a balanced diet (Bender, 1992). However, because of its nutritional qualities, meat is a very favorable ground for most microbial contamination (Bender, 1992).

Meat and meat products are ranked among the foods, most involved in collective food-borne outbreaks (TIAC) in Algeria (Mouffok, 2011). These diseases are responsible for serious health problems worldwide and the World Health Organization (WHO) estimates that, diarrhea kills 1.5 million people, and 70% of these cases

are attributed to food consumption. In addition, food-borne outbreaks have a great social and economic impact: work stoppages, stoppages of agribusiness companies and restaurants involved, which can go as far as bankruptcy, court costs, bacteriological analysis and medical costs (Corpet, 2014).

In Algeria, the treatment of a banal poisoning costs the state around 3000 DA. This figure will increased in case of hospitalization and reaches a minimum of between 20,000 and 30,000 DA per day (Maïdi, 2012).

This study investigates bacterial contamination by assessing and counting coagulase positive staphylococci and taking the storage temperature of certain meat products, widely consumed in Algeria in three different sales outlets (independent butchers, covered markets and supermarkets), in order to assess their risks to public health.

MATERIALS AND METHODS

Sampling

A total of 25 samples of meat products from bovine red meat (minced meat, Merguez) and white meat (turkey minced, chicken) were studied. The samples were taken randomly between April and June 2015 from two different communes: Bab Ezzouar and Mohammadia. Three types of trade were targeted: independent butchers, covered markets and supermarkets.

The storage temperature of these meat products was recorded using a digital food thermometer (thermometer for food use) for each sample taken. The samples (200 g) were packaged in sterile bags, clearly identified and kept in a cooler under cold conditions. These bags were immediately sent to the veterinary and quality control laboratory (AVCQ-LAB) of Algiers. Their contents were analyzed as soon as they arrived at the laboratory.

Search and enumeration of coagulase positive staphylococci

In the laboratory, 10 g of each sample was placed in sterile stomacher bags. Then, 90 ml of the PSE diluent (Peptone, salt, water) (Institut Pasteur, Algeria) was introduced into the bags. The whole was crushed for 2 min in the stomacher to thereby make the stock solution 10^{-1}. The supernatant obtained after the grinding was recovered in a sterile flask. The latter was left to stand for 45 min, to allow the revivification of the stressed bacteria.

Coagulase positive staphylococci (CPS) were grown on Baird Parker agar (Institut Pasteur, Algeria) supplemented with egg yolk and potassium tellurite. The bacterial number is evaluated after 48 h of incubation at 37°C and their identity is confirmed by the search for catalase on H_2O_2 and coagulase on the plasma of rabbit (Arrêté ministériel, 2014).

The results were compared with the criteria required by interministerial decree N°35-1998 of 24 January 1998 on the quality of foodstuffs. The maximum accepted concentrations for the bacteria counted are: 2 $\log_{10}$ cfu/g for minced meat and Merguez, 2.70 $\log_{10}$ cfu/g for turkey and chicken (Arrêté interministériel, 1998).

Statistical analysis

The average bacterial burdens were calculated by type of trade and type of levy. The type of trade (independent butchers, covered markets and supermarkets) and storage temperature were used as a source of variation.

A factorial analysis of variance (ANOVA) was used to compare the results of coagulase positive staphylococci means between the three types of trade. It is also used to compare the means of enumeration of these bacteria according to the type of sampling.

The correlation coefficient (r) was calculated between the coagulase positive staphylococci means and the storage temperature of the four meat products at the time of sampling. The Student test was used to compare the mean number of bacterial colonies with the acceptability threshold for coagulase positive staphylococci. It was thus used for comparison between the two types of meat.

Before carrying out the statistical analysis, the first step was to test normality. To do this, the most powerful test is that of Shapiro-Wilk.

All calculations were carried out using the free statistical software of the latest version (R 3.3.3 of March 2017), after transformation decimal logarithmic of the results expressed as cfu/g to normalize the distribution.

RESULTS

Overall bacteriological quality of the meat products

Table 1 reports the concentration of coagulase positive staphylococci according to the meat product considered, expressed as $\log_{10}$ cfu/g. In the light of the results obtained, Merguez (2.02 ± 0.46 $\log_{10}$ cfu/g) and turkey (2.02 ± 0.47 $\log_{10}$ cfu/g) are more likely to be loaded with coagulase positive staphylococci compared to the minced meat (1.60 ± 0.33 $\log_{10}$ cfu/g) and chicken (1.63 ± 0.21 $\log_{10}$ cfu/g).

Depending on meat quality, Merguez (2.02 ± 0.46 $\log_{10}$ cfu/g) is more contaminated with coagulase positive staphylococci compared to minced meat (1.60 ± 0.33 $\log_{10}$ cfu/g) in the case of red meats. At the same time, turkey (2.02 ± 0.47 $\log_{10}$ cfu/g) is more contaminated with coagulase positive staphylococci than chicken (1.63 ± 0.21 $\log_{10}$ cfu/g) in the case of white meat.

The interpretation of the results obtained according to the thresholds of acceptability, set by interministerial decree N°35, 1998, respectively gave non-conformity rate of 20.0% for minced meat and 41.7% for Merguez. For other products (turkey and chicken), the enumeration results are below the threshold set by the Algerian regulations (2.70 $\log_{10}$ cfu/g), therefore they are 100% compliant (Arrêté interministériel, 1998).
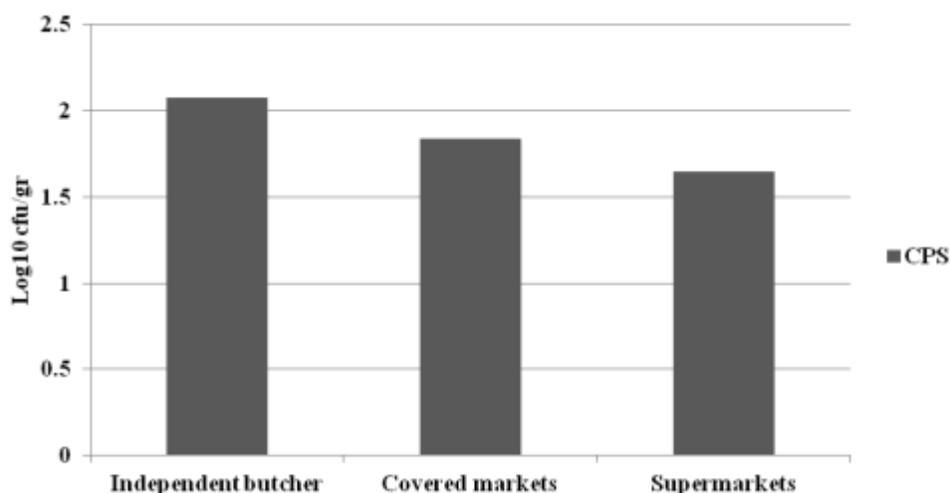
The comparison between the averages of the coagulase positive staphylococci with the acceptability threshold for each type of sampling showed a non-significant difference (p > 0.05) for minced meat and Merguez, and a significant difference (p < 0.05) for turkey and chicken. The statistical analysis (ANOVA) revealed a non-significant difference (p > 0.05) between the bacterial composition of coagulase positive staphylococci of the four foodstuffs (minced meat, Merguez, turkey and chicken).

Comparison of coagulase positive staphylococci enumeration results for the two types of meats studied

Table 1. Variation in the amount of coagulase positive staphylococci of the meat products analyzed (Mean ± SD in $\log_{10}$ cfu/g).

Variable	Red meat		Mean	White meat		Mean
	Minced meat	Merguez		Turkey (minced)	Chicken	
CPS	1.60 ± 0.33	2.02 ± 0.46	1.89 ± 0.46	2.02 ± 0.47	1.63 ± 0.21	1.92 ± 0.44
PS<CR (%)	80.0%	58.33%	64.7%	100%	100%	100%
PS>CR (%)	20.0%	41.7%	35.3%	0%	0%	0%

CPS: Coagulase positive staphylococci; PS<CR: Percentage of samples which present a bacterial burden lower than the criterion fixed by Algerian standards; PS>CR: Percentage of samples which present a bacterial load higher than the criterion fixed by the standard; (%): Prevalence.

Figure 1. Distribution of coagulase positive staphylococci according to business type.

showed a non-significant difference (p > 0.05) between the bacterial load of red and white meats.

Distribution of the results according to the type of business

According to the results, meat products from independent butcheries (2.08 ± 0.40 $\log_{10}$ cfu/g) are more heavily loaded by coagulase positive staphylococci than those on the covered markets (1.84 ± 0.45 $\log_{10}$ cfu/g) and that of supermarkets (1.65 ± 0.44 $\log_{10}$ cfu/g) (Figure 1).
Statistical analysis showed a non-significant difference (p > 0.05) between the three types of trade.

Relationship between coagulase positive staphylococci contamination and the storage temperature of meat products at the point of sale

According to meat products, the storage temperature recorded in the various sites interacted with the contamination processes. From a minimum of, 7.40°C for minced meat, 2.90°C for Merguez, 7.20°C for turkey and

8.30°C for chicken while the maximum 22.5°C for minced meat, 20.5°C for Merguez, 13.2°C for turkey and 9.10°C for chicken. Mean storage temperatures were 13.7 ± 5.90°C for minced meat, 11.2 ± 5.87°C for Merguez, 11.3 ± 2.36°C for turkey and 8.70 ± 0.57°C for chicken (Figure 2).
The storage temperatures were very close to the Algerian standard (+4°C) at the level of supermarkets.On the other hand, the majority of the samples made in covered markets and independent butchers far exceeded the temperature recommended by the Algerian standards.
The statistical analysis shows that coagulase positive staphylococci have a development with a slightly weak correlation for minced meat (r = 0.48, R² = 0.23), large correlations for Merguez (r = 0.04, R² = 0.002) and the other for turkey (r = -0.02, R² = 0.0002) (Table 2).

DISCUSSION

The meat products selected from the present work are products which have been manipulated during their preparation, thus allowing evaluation of the contamination
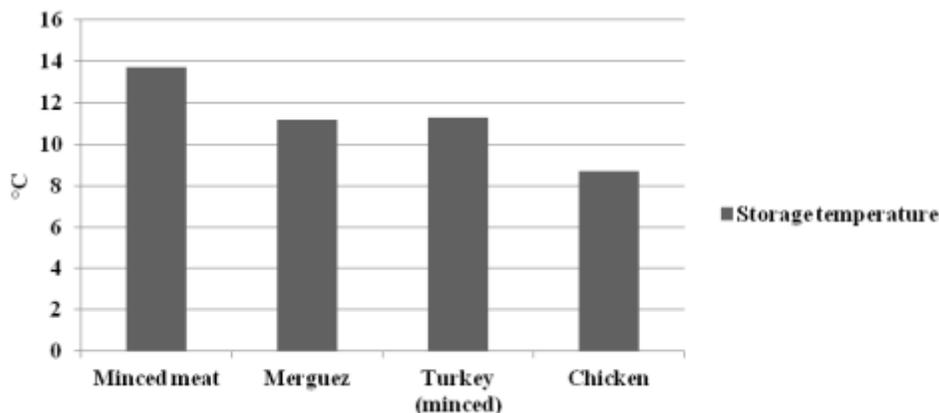
Figure 2. Distribution of the storage temperature according to the type of sampling.

Table 2. Correlation between the coagulase positive staphylococci enumeration results and the storage temperature.

Type of sampling	Relationship between the parameters	r	R^2
Minced meat	CPS-TS	0.48	0.23
Merguez	CPS-TS	0.04	0.002
Turkey (minced)	CPS-TS	-0.02	0.0002
Red meat	CPS-TS	0.03	0.0008
White meat	CPS-TS	0.21	0.05
General	CPS-TS	0.05	0.002

CPS: Coagulase positive staphylococci; TS: Temperature of storing; r: Coefficient of correlation; R^2: Coefficient of determination.

by human manipulation which adds to the initial contamination. In addition, three different types of sales outlets were targeted to assess the degree of contamination according to the hygiene status of each type of trade.

Coagulase positive staphylococci are considered to be pathogenic bacteria and their presence in food is due to poor handling conditions during preparation as well as poor hygienic quality of the material used in the food chain (Salihu et al., 2010).

The results obtained have an average of 1.60 ± 0.33 $\log_{10}$ cfu/g for minced meat, 2.02 ± 0.46 $\log_{10}$ cfu/g for Merguez, 2.02 ± 0.47 $\log_{10}$ cfu/g for turkey and 1.63 ± 0.21 $\log_{10}$ cfu/g for chicken. The frequency of results exceeding the legal criterion is 20.0% for minced meat and 41.7% for Merguez. For meat products derived from white meat (turkey, chicken), the enumeration results are below the threshold, set by the Algerian regulations (2.70 $\log_{10}$ cfu/g), that is they are 100% compliant. These results (1.60 ± 0.33 $\log_{10}$ cfu/g) are clearly inferior than those of Bouzid et al. (2015), who reported an average level of contamination of 4.61 ± 1.41 $\log_{10}$ cfu/g for fresh minced meat.

In a similar study, Chaalal (2013) confirmed the isolation of 55.5, 60 and 20% strains of *Staphylococcus aureus* from minced meat and Merguez samples, respectively, in Tiaret region (Algeria), while in the study conducted by Cohen et al. (2006) on Moroccan, meat products taken from different places of sale, *S. aureus* was detected in 16% of the samples distributed in red meat and offal, poultry meat, bovine minced meat, fresh sausages and fishery products. Whereas Sebban (1995) detected higher rates ranging from 33 to 52% (between 10^2 and 10^6 cfu/g) out of a total of 133 samples including raw, bovine, equine, cooked minced meat and fresh sausages of the Merguez type, obtained from different places of preparation or sale of the city of Rabat (butchers, supermarkets, restaurants). These results are similar to those detected by Normanno et al. (2005) in Italy, particularly for minced meat at a rate of 31.2%, which shows the importance of contamination of meat products by *S. aureus* both in developing countries (Algeria, Morocco) than in industrialized countries such as Italy.

Depending on the type of trade, the majority of samples taken from supermarkets recorded a low level of

contamination (1.65 ± 0.44 $\log_{10}$ cfu/g) compared with independent butcheries (2.08 ± 0.40 $\log_{10}$ cfu/g) and the covered markets (1.84 ± 0.45 $\log_{10}$ cfu/g), which can be explained by the observations taken at the time of sampling. It was noted that the hygiene conditions in these places of sale were better than those noted in independent butcher shops and those located in the covered markets.

In these modern supermarkets, staff wears suitable outfits, including gloves, cutting rooms and worktops, which were clean and equipped with a knife sterilizer and non-manual hand wash. The products were served in food trays and wrapped with a cellophane film. In addition, the checkout place is separated from the place of preparation, which may reduce the contamination.

The storage temperature of foodstuffs plays a major role in bacterial growth. Our results show that the majority of samples taken from covered markets and independent butchers exceeded by far temperature of 10°C. This temperature is considered as a threshold from which the *S.aureus* bacterium can start producing the enterotoxin responsible for the disease (Hennekinne, 2009). On the other hand, storage temperatures were very close to the norm at the level of supermarkets.

The correlation between the coagulase positive staphylococci contamination level of the four meat products and the storage temperature of these products showed a slightly weak correlation for minced meat (r = 0.48) and two other largely weak correlations, one positive for Merguez (r = 0.04) and the other negative for turkey (r = -0.02).

According to De Buyser (1996), coagulase positive staphylococci multiply at temperatures between 6 and 46°C with an optimal temperature of 37°C and toxinogenesis occurs under conditions somewhat more restrictive than those required for growth.

Conclusion

The results of this study confirm the importance of contamination of meat products by coagulase positive staphylococci in different places of sale (independent butchers, covered markets and supermarkets). Contamination by these bacteria is considered as an important indicator of hygiene, since the contamination can be of human origin during the manufacture of food or its domestic preparation.

In addition, this work sheds light on the conditions of conservation of these products by evaluating the temperature of storage at the point of sale. This factor is primordial in staphylococcal foodborne disease. However, a change can be noticed in practice food of Algerians and a development of the sector of the fast food.

Therefore, it is important to respect the cold chain and hygienic conditions when preparing meat products. Sensitization and popularization of consumers and other stakeholders in the food chain on the dangers of eating and preparing meat products is therefore necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Arrêté interministériel (1998). Relatif aux spécifications microbiologiques de certaines denrées. J. Off. Répub. Algérienne. Démocr. 35:7-24.

Arrêté ministériel (2014). Rendant obligatoire la méthode de dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces). J. Off. Répub. Algérienne. Démocr. 68:17–22.

Bender A (1992). Meat and meat products in human nutrition in developing countries. Animal Production and Health Division and the Food Policy and Nutrition Division of FAO, Rome. 53p.

Bouzid R, Guemour D, Zidane K, Aggad H, Bendella A, Saegerman C (2015). Hygienic quality of minced meat retailed in western Algeria. J. Virol. Microbiol. 2015:c1-9.

Chaalal W (2013). Occurrence et profil d'antibiorésistance des *Staphylococcus aureus* isolés de produits alimentaires. Thèse Magister, Univiversité Es-senia, Oran. 94p.

Cohen N, Ennaji H, Hassar M, Karib H (2006). The bacterial quality of red meat and offal in Casablanca (Morocco). Mol. Nutr. Food. Res. 50*(6)*:557-562.

Corpet D (2014). Cours HIDAOA, les Toxi-infection Alimentaires collectives [En ligne]. Adresse URL: http://Corpet.net/Denis.

De Buyser ML (1996). Les staphylocoques. In : Bourgeois C., Mescle J.F. (Eds), Microbiologie alimentaire. Tome 1. Lavoisier: Paris. pp. 106-119.

Hennekinne JA (2009). Nouvelles approches pour la caracterisation des toxi infections alimentaires a staphylocoques a coagulase positive. Thèse Doctorat, Institut des Sciences et Industries du Vivant et de l'Environnement, Agro Paris Tech. 183p.

Maïdi A (2012). L'Observatoire national de la sécurité alimentaire sera-t-il un jour opérationnel?. El Watan du samedi 6 octobre 2012.

Mouffok F (2011). Situation en matière de TIA en Algérie de 2010 à 2011. 2ème congrès Maghrébin sur les TIA, 14 - 15 décembre 2011, Tunis.

Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Decastelli L, Mioni R, Scuota S, Bolzoni G, Di Giannatale E, Salinetti AP, La Salandra G, Bartoli M, Zuccon F, Pirino T, Sias S, Parisi A, Quaglia NC, Celano GV (2005). Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. Int. J. Food. Microbiol. 98 (1):73- 79.

Salihu MD, Junaidu AU, Magaji AA, Aliyu RM, Yakubu Y, Shittu A, Ibrahim MA (2010). Bacteriological quality of traditionally prepared fried ground beef (Damb*unnama*) in Sokoto, Nigeria. Adv. J. Food. Sci. Technol. 2(3):145-147.

Sebban Z (1995). Evaluation des contaminations par *Staphylococcus aureus* de certains produits carnés marocains. Thèse DES, Faculté des Sciences, Université Cadi Ayyad, Marrakech. http://toubkal.imist.ma/handle/123456789/7912.