# Accepted Manuscript


Prevalence, genetic characterization and biofilm formation in *vitro* of *staphylococcus aureus* isolated from raw chicken meat at retail level in Nanjing, China

Huawei Wang, Huhu Wang, Lijiao Liang, Xinglian Xu, Guanghong Zhou

| | |
|---|---|
| PII: | S0956-7135(17)30513-3 |
| DOI: | 10.1016/j.foodcont.2017.10.028 |
| Reference: | JFCO 5837 |
| To appear in: | *Food Control* |
| Received Date: | 26 July 2017 |
| Revised Date: | 22 October 2017 |
| Accepted Date: | 23 October 2017 |

Please cite this article as: Huawei Wang, Huhu Wang, Lijiao Liang, Xinglian Xu, Guanghong Zhou, Prevalence, genetic characterization and biofilm formation in *vitro* of *staphylococcus aureus* isolated from raw chicken meat at retail level in Nanjing, China, *Food Control* (2017), doi: 10.1016/j.foodcont.2017.10.028

Highlights

Prevalence and population of *S. aureus* in different raw chicken meats determined.

Cuts and unpacked products showed relatively higher contamination of *S. aureus*.

High homogenous genotypes acquired among various products.

The low toxic gene existence suggested low potentiality of SFP.

Presence of strong biofilm formation and 48% MDR needed to be highly concerned.

# Prevalence, genetic characterization and biofilm formation in *vitro* of *staphylococcus aureus* isolated from raw chicken meat at retail level in Nanjing, China

Huawei Wang[a], Huhu Wang[b], Lijiao Liang[a], Xinglian Xu[a]*, Guanghong Zhou[a,b]

[a] *National Center of Meat Quality and Safety Control, Nanjing Agricultural University, Nanjing 210095, P.R. China*

[b] *Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, Nanjing Agricultural University, Nanjing 210095, P.R. China*

* Corresponding author. Tel.: +86 25 84395939; fax: +86 25 84395730.

E-mail address: xlxu@njau.edu.cn (X. Xu).

**ABSTRACT**

*Staphylococcus aureus* is widespread in animal-origin food, particularly for raw chicken meat products because of the direct exposure and cross-contamination at the breeding, slaughtering and processing, transporting, storing and marketing stages. This study aims to determine the *S. aureus* prevalence, antibiotic resistance (routine animal used and nosocomial treatment antibiotics) and genetic characterization (e.g. frequently used molecular typing methods for *Staphylococcus aureus*, enterotoxin series, panton valentine leukocidin, toxic shock syndrome) among various raw chicken meat products. In total, 464 raw chicken meat products were collected in a variety of conditions: from local supermarkets and wet markets; between summer and winter; of cut and whole carcass product types; stored at both low temperature and room temperature; and in both packed and unpacked states. The overall prevalence of *S. aureus* was 11.5%, contamination level of *S. aureus* among different products types maintained $10^2$-$10^4$ CFU/g, clonal complex (CC)5 was the most common CC in various products (74.2%); every sequence type (ST) corresponded to

a single *spa* type, remarkably, *spa* typing did not show further discrimination based on MLST. Toxin genetic distribution showed the existence of diverse atypical enterotoxin genes, *sea*, *pvl* and *tst* as classical and critical toxin factors were absent in these isolates. Whereas, penicillin- and tetracycline-resistant isolates were common in products with skin, cuts, particularly, extremely strong biofilm formation isolates were found in products with skin. Our investigation indicates that cutting process and packing were the key factors for the contamination of *S. aureus*, suggesting a possible critical control points in processing and marketing stages. Though a relatively low toxin genetic existence suggests low virulence, routine antibiotic resistance and high biofilm formation existence raise the need for concern over possibility of human infection.

**Keywords:**

*Staphylococcus aureus*; Raw chicken meat; Genetic characterization; antimicrobial resistance; biofilm formation

## 1. Introduction

*Staphylococcus aureus* is a human and animal host-specific pathogen involved in multiple diseases (Visciano, et al., 2014). It is regarded as one of the world's leading causes of food consumption-related disease outbreaks (Schelin, et al., 2011). Enterotoxic *S. aureus* produces multiple heat-stable extracellular enterotoxins which may lead to pathogenicity in Ready-To-Eat (RTE) foods and Ready-To-Cook (RTC) foods, common symptoms of Staphylococcal food poisoning (SFP) are vomiting, diarrhea, abdominal cramping and exhaustion (Ertas Onmaz, et al., 2015; Normanno, et al., 2005; Puah, Chua, & Tan, 2016; Song, et al., 2015). Currently, documented food-borne outbreaks of *S. aureus* intoxications are mostly associated with consumption of contaminated food (Fetsch, et al., 2014; Kerouanton, et al., 2007).

Raw chicken meat products as the worldwide leaders in consumed RTC meat are common in slaughterhouses of all sizes (Abdalrahman, Stanley, Wells, & Fakhr, 2015), because of the daily massive consumption, these products constitute a high potentiality of SFP through contamination in further processing. As such, raw chicken meat products are uniquely predisposed to becoming vehicles for potential transmission of pathogenic *S. aureus* to humans via food chain. Moreover, the amount of pathogenic *S. aureus* recovered from food remnants is generally recognized to require a presence greater than $10^5$ CFU/g to cause SFP (Fetsch, et al., 2014), depending on the toxigenicity of the *S.aureus* strain, initial contamination level in processing units, transportation time and temperature, and the environmental conditions of terminal marketing (Soriano, Font, Moltó, & Mañes, 2002). In addition, regarding public infections, multidrug resistance (MDR) is an emerging concern among all kinds of food-origin meat (Petternel, et al., 2014), especially for antibiotics abuse at feeding stage.

*S. aureus* presents an extensive sequence typing diversity associated with seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi and yqiL*), Staphylococcal protein A (*spa*), and virulence genetic diversity such as staphylococcal enterotoxins (*se*), toxic shock syndrome toxin (*tst*), extracellular thermo-stable nuclease (*nuc*), β-lactamase (*bla*), staphylococcal cassette chromosome *mec* (*SCCmec*), and accessory gene regulators (*agr*) (Argudin, et al., 2012; Carfora, et al., 2015; de Boer, et al., 2009; Omoe, et al., 2002; Zhang, et al., 2011). Enterotoxin genes acquired through inheritance or horizontal transformation, form pathogenic islands grouped as enterotoxin gene clusters (*egc*) or organized as an operon. Additionally, antimicrobial resistance is conditionally acquired via plasmids or thansposons carrying antibiotic resistance genes or mutations, especially methicillin-resistant *S. aureus* (MRSA) harboring methcillin-resistant (*mec*) gene, can be grouped

according to *SCCmec* typing (Song, et al., 2015).

Therefore, it is important to investigate the prevalence, antimicrobial resistance and genetic diversity of *S. aureus* in chicken meat products at a retail level among diverse marketing and storage conditions. The basic data of *S. aureus* contamination in raw chicken meat products would contribute to improve the microbial quality of such products. Samples were classified based on different markets, brands (including nameless and well-known brands), cutting and packing types to determine their influence on the contamination of *S. aureus*.

## 2. Materials and Methods

### 2.1. Sample collection

Out of 464 raw chicken meat samples were collected from randomly selected supermarkets and wet markets in Nanjing, China, Samples were chosen with the aim of obtaining a variety of brands, markets, cuts (breast, wings, drumsticks and whole carcass) and storage conditions. In supermarkets, raw chicken meat products were stored on ice, and in refrigerators with or without tray packing. In wet markets, unpacked raw chicken meat products were frozen or stored at room temperature (RT). Each sample was transported to the laboratory on ice in sterilized bag in order to avoid cross-contamination.

### 2.2. Quantification of *S.aureus*

Quantification of coagulase-positive staphylococci (CPS) was performed as described by China's National food Safety Standard-Food for microbiological examination of *S. aureus* (GB4789.10-2010). Briefly, 25 g of product was mixed with 225 ml sterilized saline and homogenized in a stomacher masticator, then homogenates were serially diluted with sterilized saline. Subsequently, 0.3 ml, 0.3 ml and 0.4 ml of each dilution was streaked onto Baird Parker

agar with 5% egg yolk and tellurite emulsion (Beijing Land Bridge Tech Co., Ltd., China), thereafter, 2 or 3 consecutive dilutions were chosen for each sample, with 37℃ cultivation for 48 h. Typical *S. aureus* colonies based on morphology were counted for calculation.

The formulae for quantification were as described below:

*a*- Only one dilution had colonies in consecutive dilutions.

*b*- The low dilution selected has more than 200 colonies, while following high dilutions have fewer than 20 colonies.

The two above conditions accorded:

$$T = {}^{AB}/_{C}d \quad (1)$$

where T is *S. aureus* colonies in sample, A is typical colonies at one dilution, B is coagulase positive colonies at one dilution, C is colonies subjected to coagulase test at one dilution, and *d* is the dilution factor.

*c*- Consecutive dilutions both had 20-200 colonies accord:

$$T = (A1B1/C1 + A2B2/C2)/1.1d \quad (2)$$

where T is *S. aureus* colonies in sample, A1 and A2 are typical colonies at low dilution and high dilution respectively, B1 and B2 are coagulase positive colonies at low dilution and high dilution respectively. C1 and C2 are colonies subjected to coagulase test at low dilution and high dilution respectively, 1.1 is the coefficient, and *d* is the dilution factor (low dilution).

2.3. Identification of *S. aureus*

For further exact identification of *S. aureus*, 3–5 presumptive colonies on each plate were picked to inoculate TSB medium for overnight culturing at 37℃, carried out with VITEK2 automated system (BioMerieux, France), and hemolytic and coagulase test (Beijing Land Bridge

Technology LTD., China). Genomic DNA of *S. aureus* isolates were extracted and purified using commercial bacteria DNA extraction and purification kits (Tiangen Biotech Co., Ltd., China). *S. aureus* genus specific primers (multiplex PCR targeting *nuc*1, *coa* and staphylococcal specific 16S rRNA) were used for molecular identification (Perillo, et al., 2012).

2.4. Molecular typing

PCR for Multi-Locus Sequence Typing (MLST) and Staphylococcal protein A (*spa*) typing of recovered *S. aureus* were performed according to public primers (synthesized by Sango Co., Ltd., China) and procedures. Amplicons were sequenced followed by sequences alignment on the MLST website (http://www.mlst.net) and Ridom Spa Server (http://spaserver.ridom.de/) separately. Further analysis for clonal complexes (CC) of allelic types was also defined as described previously (Normanno, G., 2015). In addition, *agr* genotyping was carried out as described by (Song, et al., 2015), and staphylococcal cassette SCC*mec* types for methicillin-resistant *Staphylococcus aureus* (MRSA) were analyzed using multiplex PCR as described previously (Zhang, et al., 2011).

2.5. Virulence and antimicrobial resistance genetic characterization

Virulence and antimicrobial resistance genetic characterization of *S. aureus* isolates were screened by simplex PCR assay for eighteen enterotoxins (*sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq, ser, seu*), panton-valentine leucocidin (*pvl*), exfoliatins (*eta*, *etb*, *etd*), toxic shock syndrome toxin (*tst*), and penicillin (*blaZ*) and methicillin resistance (*mecA*). The colistin resistant gene *mcr*-1 was also considered for its horizontal transfer potentiality in wild complex microfora (Liu, et al., 2016). Primers used are listed in Table 1. Amplicon of each positive toxin gene sequenced to confirm PCR accuracy.

2.6. Antimicrobial susceptibility

Out of 12 antimicrobial agents were selected for standardized disc diffusion test on Muller-Hinton agar. These commercial discs were vancomycin (30 μg), chloromycetin (30 μg), clindamycin (2 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), erythromycin (15 μg), penicillin (10 U), oxacillin (1 μg) and spectinomycin (100 μg). ATCC 25923 was used as quality control. Breakpoints were established for each antibiotic according to the Clinical and Laboratory Standards Institute (CLSI, 2014), and MDR (Multi Drug Resistance) was defined as three or more classes of antibiotic resistance or methicillin resistance (Petternel, et al., 2014).

2.7. Biofilm formation in *vitro*

The biofilm-forming ability (BFA) was assayed using TSB culturing in polystyrene microtiter plates, followed by crystal violet dying procedure adhering to standards set out in a previous report (Vázquez-Sánchez, Cabo, Ibusquiza, & Rodríguez-Herrera, 2014) with slight modification. Culture condition was 37℃ for 48 h, optimal density (OD) at 595 nm wavelength was measured at the end time point, and ATCC 6538 was used as biofilm positive control (Peeters, Nelis, & Coenye, 2008). Three independent experiments were performed in triplicate. BFA was assayed using formulae based on previously reports (Ruiz, Barragan, Sesena, & Palop, 2016):

$$\mathrm{BFA} = (a - c)/(b - c) \quad (3)$$

where strong BFA was defined as value ＞2; $a$, $b$ and $c$ are OD values of isolates, ATCC 6538 and blank well as positive and negative control respectively.

2.8. Statistical analysis

The quantification of CPS was graphed using GraphPad prism 5.0 (GraphPad Software, Inc.,

USA),SPSS 17.0 were employed for statistical analysis as follow: a Pearson's chi-square test was carried out for comparison of prevalence of CPS, one-way ANOVA was used to test the significant difference of quantification of CPS. eBUST v.3 was used for locus variant analysis by allelic types of MLST, and the Fisher exact test was used for genetic distribution among different products categories. Significant difference was determined at a 5% confidence interval.

## 3. Results

### 3.1. Prevalence and quantification of CPS

As shown in Table 2, out of 464 raw chicken meat products collected from local supermarkets (288) and wet markets (176), 53 (11.5%) samples showed the presence of CPS as determined by BP-EY agar, hemolytic and coagulase tests. A Chi-squared test of independence indicated that the overall prevalence of CPS in supermarkets (37/288) and in wet markets (16/176) did not differ significantly ($p>0.05$). Because there was no significant difference between them, samples from supermarkets and wet markets were combined for further analysis. The prevalence of CPS did not differ significantly ($p>0.05$) between packed and unpacked products, or between those stored at room temperature and low temperature. However, there was a significant difference ($p<0.05$) between products with skin (drumstick, wings and whole carcass products) and products without skin, also between cuts (drumstick, wings and breast) and whole carcass products.

According to China's National Food Safety Standard-Food microbiological examination of *S. aureus* (GB4789.10-2010), the population of *S. aureus* was quantified as a range of different values for each different categories of raw chicken meat. As showed in Fig.1, all types remained the contamination level range from$10^2$ to $10^4$ CFU/g. comparatively, Refrigerated types had significant lower contamination compared to RT stored products ($p<0.05$), among them,

unpacked types showed significant higher contamination compared to packed ones ($p<0.05$). Nevertheless, RT stored types were only sold in wet markets, the influence of setting cannot be differentiated between supermarkets and wet markets, moreover, cuts and whole carcass, products with and without skin had no significant difference in various contamination levels.

3.2. Genotypes

Previous studies which have characterized *S. aureus* isolates from food-related materials have rarely regarded the overlap among these isolates (Merz, Stephan, & Johler, 2016; Normanno, et al., 2007; Perillo, et al., 2012).For several typical colonies using the method of molecular distribution on a single plate, the only discrimination made was antibiotics susceptibility profiles (Perillo, et al., 2012). To avoid repeats during calculation, our study defined a single *S. aureus* isolate based on ST, *spa* type, *agr* type, antimicrobial resistance and virulence genes profiles. Totally, 31 single strains were determined among 53 CPS, as shown in Table 3, 31 isolates grouped into six MLST types. The most prevalent genotype was ST1 (16/31), followed by ST12 (6/31), ST2315 (3/31), ST5 (2/31), ST7 (2/31) and ST8 (2/31). eBURST v.3 analysis showed that ST1, ST5, ST8 and ST2315 were grouped into one CC5, but ST7 and ST12 were solely founded by CC7 and CC12 respectively. Every ST corresponded to a single *spa* type. ST1 corresponded to t127, ST5 to t002, ST7 to t091, ST8 to t9101, ST12 to t213 and ST2315 to t11687. In addition, *agr* typing revealed that, Ⅰ Ⅱ and Ⅲ types were identified in isolates except type Ⅳ, Ⅲ type was the most common *agr* type (16/31), followed by type Ⅱ (11/31) and type Ⅰ (8/31).

Regarding genotypes distribution among different products types, since each ST type had consistent one-to-one match with *spa* type, the distribution of genotypes were considered based on CC. Overall, isolates from diverse product types exhibited a high degree of heterogeneity, the

existence of CC5 were found in every product type. Cuts had higher existence of CC5 compared with whole carcasses; same results were obtained in packed types compared with unpacked types, products with or without skin, refrigerated and RT stored types. CC12 was mainly distributed in refrigerated and unpacked types.

For toxic gene distribution, *seh* (16/31), *sec* (10/31) and *sel* (10/31) had relatively higher existence among isolates, compared with other enterotoxins genes *seb, seg, sei, sem, sen* and *seo*(all less than 5/31). Notably, *sea*, *tst* and *pvl* as classical toxin genes were absent in these isolates, *sek*, *seq* or *seu* were absent neither. Meanwhile, common presence of *bla* (27/31) geneand absence of *mec* gene confirmed the antibiotic susceptibility. For toxic gene distribution in products categories, regarding combined calculation of prevalence of CPS, both number and diversity of toxin genes in cuts or low temperature products were shown to be significantly higher than whole chicken or room temperature products, especially for *sec*, *seh*, and *sel*.

### 3.3. Antimicrobial susceptibility

Antimicrobial susceptibility testing showed that all *S.aureus* isolates were susceptible to gentamycin, oxacillin, cefaclor and vancomycin. Antibiotics Resistance associated with product types are shown in Table 4, 27 (87.1%) and 26 (83.9%) of 31 isolates were resistant to penicillin and tetracycline respectively, followed by 11 (35.5%) isolates resistant to ciprofloxacin, 10 (32.3%) to erythromycin. Only several isolates were resistant to streptomycin (9.7%), chloromycetin (9.7%), clindamycin (3.2%) and kanamycin (3.2%). In addition, 15 (48.4%) isolates showed MDR phenotypes like TC-P-CIP, TC-P-E, TC-CIP-E, TC-P-CIP-E and S-TC-P resistance.

A combined comparison was carried out in products categories, it showed that tetracycline and

221 erythromycin resistance rates are significantly higher in skin, cuts and low temperature stored
222 products compared with breast, whole carcass and room temperature products respectively. The
223 existence of penicillin resistance was significantly higher in skin and low temperature products
224 than in breast and room temperature products; however, other antibiotic resistances were
225 randomly distributed because of low performance of resistance.

226 3.4. Biofilm formation ability

227 As shown in Table 5, six isolates showed strong BFA, while the BFA of other isolates were
228 either similar to ATCC 6538 or biofilm-forming negative (data not shown). Among them, two
229 isolates (Sa02 and Sa05) had extremely strong BFA which were both isolated from chicken meat
230 products with skin, found in supermarket and wet market respectively.

231 **4. Discussion**

232 Studies of *S.aureus* related to food generally focus on fish, dairy, poultry, livestock and
233 handling contacts (Carfora, et al., 2015; de Boer, et al., 2009; Li, Wu, Wang, & Meng, 2015;
234 Lozano, Gharsa, Ben Slama, Zarazaga, & Torres, 2016; Normanno, et al., 2005; Normanno, et al.,
235 2007; Simon & Sanjeev, 2007; Yan, et al., 2012). In the past decade, though relevant reports have
236 demonstrated that the high prevalence of *S.aureus* in chicken products (Abdalrahman, et al., 2015;
237 Fijalkowski, Peitler, & Karakulska, 2016; Thapaliya, et al., 2017), different processing units,
238 storage temperature, retailing environment and product types may cause a diverse contamination
239 level of *S. aureus*, which has rarely been considered in previous studies. Therefore, there is a great
240 practical significance to the investigation of the prevalence of *S.aureus* among various raw
241 chicken meat products according to different environmental conditions.

242 The results of this study indicated that different retail environments (i.e. supermarkets and wet

markets) had no significant influence on the prevalence of CPS in raw chicken meat samples. Significant difference of prevalence of CPS occurred between room temperature and low temperature stored products, same result was obtained in the quantification of CPS, presumably, this is because environmental temperature has a critical influence on the growth of bacteria. Besides, there were significant differences of prevalence of CPS between products with or without skin, cuts or whole carcass, this indicates that original contamination of CPS in processing units is the decisive influential factor in CPS contamination (Normanno, et al., 2005; Soriano, et al., 2002), particularly because of cross-contamination between handling, equipment contact surface and meat surface (Habib, et al., 2012; You, et al., 2016). The quantification of CPS among different products indicated that packing process is the main cause of significant difference of contamination level of CPS among various types. In brief, the key factors influencing contamination of *S.aureus* on raw chicken meat were cutting and packing processes, each of which was significant. This study's findings should be used to highlight processing and storing standards considering these condition changes had an effect on the microflora of meat, that is, cutting and packing process was the main influential factors because further handling processes have increased the opportunity for cross-contamination (Ertas Onmaz, et al., 2015).

Quantification of *S. aureus* revealed that *S. aureus* isolated from samples show a highly similarity of molecular typing, CC5 and t127 were widely distributed in every type of products, which is in agreement with previously report (Merz, et al., 2016), however, *spa* typing did not show a further discrimination compared with ST typing (KITAI, et al., 2005). Different sampling markets and package types had no influence on the genotype distribution among products categories. As for virulence gene detection, the prevalence of atypical enterotoxins, and the

265 absence of classical toxin genes including *sea, pvl* and *tst*, revealed a relatively less toxigenicity
266 compared with previous reports associated with chicken meat products (Abdalrahman, et al.,
267 2015; Fijalkowski, et al., 2016; Lozano, et al., 2016; Song, et al., 2015; Wang, et al., 2013).

268 As for phenotypic characterization of antimicrobial resistance and BFA, Antimicrobial
269 susceptibility tests showed a general penicillin and tetracycline resistance and frequent MDR,
270 which is in agreement with previous studies (Jamali, Paydar, Radmehr, Ismail, & Dadrasnia, 2015;
271 Puah, et al., 2016; Thapaliya, et al., 2017), suggested an increasing common penicillin and
272 tetracycline resistance in *S. aureus* isolated from food-related samples worldwide. More
273 importantly, the presence of nearly half of isolates showed MDR and extremely strong BFA
274 isolates need to be highly concerned regarding the possibility of severe infections.

275 In summary, low prevalence and virulence genetic existence of *S. aureus* among different
276 chicken meat products at retail level was mostly attributed to the holistic improvements in the
277 handling and sanitary procedures (Bai, Ma, Yang, Zhao, & Gong, 2007), and adaptation of Good
278 Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) in
279 processing units (Jin, Zhou, & Ye, 2008; Soriano, et al., 2002; Tompkin, 1994). In addition, the
280 wide application of cold chain logistics conception (particularly with RFID [Radio Frequency
281 Identification] technology) ensured the effective control of microbial growth from processing
282 units to terminal marketing (Raab, et al., 2008; TANG & QIAN, 2008). These developed
283 decontamination and hygienic strategies provided an efficient microbial control among the whole
284 poultry meat processing chain, guaranteeing a low homogeneous *S. aureus* contamination in raw
285 chicken meat products.

286 **Acknowledgments**

287 This project was supported by the China Agricultural Research System (CARS-42).

## References


Abdalrahman, L. S., Stanley, A., Wells, H., & Fakhr, M. K. (2015). Isolation, Virulence, and Antimicrobial Resistance of Methicillin-Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA) Strains from Oklahoma Retail Poultry Meats. *Int J Environ Res Public Health, 12*(6), 6148-6161.

Argudin, M. A., Mendoza, M. C., Gonzalez-Hevia, M. A., Bances, M., Guerra, B., & Rodicio, M. R. (2012). Genotypes, exotoxin gene content, and antimicrobial resistance of Staphylococcus aureus strains recovered from foods and food handlers. *Appl Environ Microbiol, 78*(8), 2930-2935.

Bai, L., Ma, C.-l., Yang, Y.-s., Zhao, S.-k., & Gong, S.-l. (2007). Implementation of HACCP system in China: A survey of food enterprises involved. *Food Control, 18*(9), 1108-1112.

Carfora, V., Caprioli, A., Marri, N., Sagrafoli, D., Boselli, C., Giacinti, G., Giangolini, G., Sorbara, L., Dottarelli, S., Battisti, A., & Amatiste, S. (2015). Enterotoxin genes, enterotoxin production, and methicillin resistance in Staphylococcus aureus isolated from milk and dairy products in Central Italy. *International Dairy Journal, 42*, 12-15.

de Boer, E., Zwartkruis-Nahuis, J. T., Wit, B., Huijsdens, X. W., de Neeling, A. J., Bosch, T., van Oosterom, R. A., Vila, A., & Heuvelink, A. E. (2009). Prevalence of methicillin-resistant Staphylococcus aureus in meat. *Int J Food Microbiol, 134*(1-2), 52-56.

Ertas Onmaz, N., Abay, S., Karadal, F., Hizlisoy, H., Telli, N., & Al, S. (2015). Occurence and antimicrobial resistance of Staphylococcus aureus and Salmonella spp. in retail fish samples in Turkey. *Mar Pollut Bull, 90*(1-2), 242-246.

Fetsch, A., Contzen, M., Hartelt, K., Kleiser, A., Maassen, S., Rau, J., Kraushaar, B., Layer, F., & Strommenger, B. (2014). Staphylococcus aureus food-poisoning outbreak associated with the consumption of ice-cream. *Int J Food Microbiol, 187*, 1-6.

Fijalkowski, K., Peitler, D., & Karakulska, J. (2016). Staphylococci isolated from ready-to-eat meat - Identification, antibiotic resistance and toxin gene profile. *Int J Food Microbiol, 238*, 113-120.

Habib, I., Berkvens, D., De Zutter, L., Dierick, K., Van Huffel, X., Speybroeck, N., Geeraerd, A. H., & Uyttendaele, M. (2012). Campylobacter contamination in broiler carcasses and correlation with slaughterhouses operational hygiene inspection. *Food Microbiol, 29*(1), 105-112.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., & Dadrasnia, A. (2015). Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. *Food Control, 54*, 383-388.

Jin, S., Zhou, J., & Ye, J. (2008). Adoption of HACCP system in the Chinese food industry: A comparative analysis. *Food Control, 19*(8), 823-828.

Kerouanton, A., Hennekinne, J. A., Letertre, C., Petit, L., Chesneau, O., Brisabois, A., & De Buyser, M. L. (2007). Characterization of Staphylococcus aureus strains associated with food poisoning outbreaks in France. *Int J Food Microbiol, 115*(3), 369-375.

KITAI, S., SHIMIZU, A., KAWANO, J., SATO, E., NAKANO, C., KITAGAWA, H., FUJIO, K., MATSUMURA, K., YASUDA, R., & INAMOTO, T. (2005). Prevalence and characterization of Staphylococcus aureus and enterotoxigenic Staphylococcus aureus in retail raw chicken meat throughout Japan. *Journal of Veterinary Medical Science, 67*(3), 269-274.

Li, G., Wu, C., Wang, X., & Meng, J. (2015). Prevalence and characterization of methicillin susceptible Staphylococcus aureus ST398 isolates from retail foods. *Int J Food Microbiol, 196*, 94-97.

Liu, Y.-Y., Wang, Y., Walsh, T. R., Yi, L.-X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.-F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.-H., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161-168.

Lozano, C., Gharsa, H., Ben Slama, K., Zarazaga, M., & Torres, C. (2016). Staphylococcus aureus in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent. *Microorganisms, 4*(1), 12.

Merz, A., Stephan, R., & Johler, S. (2016). Genotyping and DNA microarray based characterization of Staphylococcus aureus isolates from rabbit carcasses. *Meat Sci, 112*, 86-89.

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

Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N. C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E., & Celano, G. V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. *Int J Food Microbiol, 115*(3), 290-296.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D. L., Ueda, S., & Shinagawa, K. (2002). Detection of seg, seh, and sei genes in Staphylococcus aureus Isolates and Determination of the Enterotoxin Productivities of S. aureus Isolates Harboring seg, seh, or sei Genes. *J Clin Microbiol, 40*(3), 857-862.

Peeters, E., Nelis, H. J., & Coenye, T. (2008). Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. *J Microbiol Methods, 72*(2), 157-165.

Perillo, J., Ceccarelli, D., Spagnoletti, M., Lollai, S., Cappuccinelli, P., & Colombo, M. M. (2012). Molecular characterization of enterotoxigenic and borderline oxacillin resistant Staphylococcus strains from ovine milk. *Food Microbiol, 32*(2), 265-273.

Petternel, C., Galler, H., Zarfel, G., Luxner, J., Haas, D., Grisold, A. J., Reinthaler, F. F., & Feierl, G. (2014). Isolation and characterization of multidrug-resistant bacteria from minced meat in Austria. *Food Microbiol, 44*, 41-46.

Puah, S. M., Chua, K. H., & Tan, J. A. (2016). Virulence Factors and Antibiotic Susceptibility of Staphylococcus aureus Isolates in Ready-to-Eat Foods: Detection of S. aureus Contamination and a High Prevalence of Virulence Genes. *Int J Environ Res Public Health, 13*(2).

Raab, V., Bruckner, S., Beierle, E., Kampmann, Y., Petersen, B., & Kreyenschmidt, J. (2008). Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. *Journal on Chain and Network Science, 8*(1), 59-73.

Ruiz, P., Barragan, I., Sesena, S., & Palop, M. L. (2016). Is staphylococci population from milk of healthy goats safe? *Int J Food Microbiol, 238*, 146-152.

Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., Barker, G. C., & Radstrom, P. (2011). The formation of Staphylococcus aureus enterotoxin in food environments and advances in risk assessment. *Virulence, 2*(6), 580-592.

Simon, S. S., & Sanjeev, S. (2007). Prevalence of enterotoxigenic Staphylococcus aureus in fishery products and fish processing factory workers. *Food Control, 18*(12), 1565-1568.

Song, M., Bai, Y., Xu, J., Carter, M. Q., Shi, C., & Shi, X. (2015). Genetic diversity and virulence potential of Staphylococcus aureus isolates from raw and processed food commodities in Shanghai. *Int J Food Microbiol, 195*, 1-8.

Soriano, J. M., Font, G., Moltó, J. C., & Mañes, J. (2002). Enterotoxigenic staphylococci and their toxins in restaurant foods. *Trends in Food Science & Technology, 13*(2), 60-67.

TANG, X.-y., & QIAN, Y.-z. (2008). Situation of Meat Cold Chain Logistics in China and Development Countermeasures [J]. *Food Science, 10*, 158.

Thapaliya, D., Forshey, B. M., Kadariya, J., Quick, M. K., Farina, S., O'Brien, A., Nair, R., Nworie, A., Hanson, B., & Kates, A. (2017). Prevalence and molecular characterization of Staphylococcus aureus in commercially available meat over a one-year period in Iowa, USA. *Food Microbiol, 65*, 122-129.

Tompkin, R. (1994). HACCP in the meat and poultry industry. *Food Control, 5*(3), 153-161.

Vázquez-Sánchez, D., Cabo, M. L., Ibusquiza, P. S., & Rodríguez-Herrera, J. J. (2014). Biofilm-forming ability and resistance to industrial disinfectants of Staphylococcus aureus isolated from fishery products. *Food Control, 39*, 8-16.

Visciano, P., Pomilio, F., Tofalo, R., Sacchini, L., Saletti, M. A., Tieri, E., Schirone, M., & Suzzi, G. (2014). Detection of methicillin-resistant Staphylococcus aureus in dairy cow farms. *Food Control, 46*, 532-538.

Wang, X., Tao, X., Xia, X., Yang, B., Xi, M., Meng, J., Zhang, J., & Xu, B. (2013). Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in retail raw chicken in China. *Food Control, 29*(1), 103-106.

Yan, X., Wang, B., Tao, X., Hu, Q., Cui, Z., Zhang, J., Lin, Y., You, Y., Shi, X., & Grundmann, H. (2012). Characterization of Staphylococcus aureus strains associated with food poisoning in Shenzhen, China. *Appl Environ Microbiol, 78*(18), 6637-6642.

You, Y., Leahy, K., Resnick, C., Howard, T., Carroll, K. C., & Silbergeld, E. K. (2016). Exposure to pathogens among workers in a poultry slaughter and processing plant. *Am J Ind Med, 59*(6), 453-464.

Zhang, W., Hao, Z., Wang, Y., Cao, X., Logue, C. M., Wang, B., Yang, J., Shen, J., & Wu, C. (2011). Molecular characterization of methicillin-resistant Staphylococcus aureus strains from pet animals and veterinary staff in China. *The Veterinary Journal, 190*(2), e125-e129.

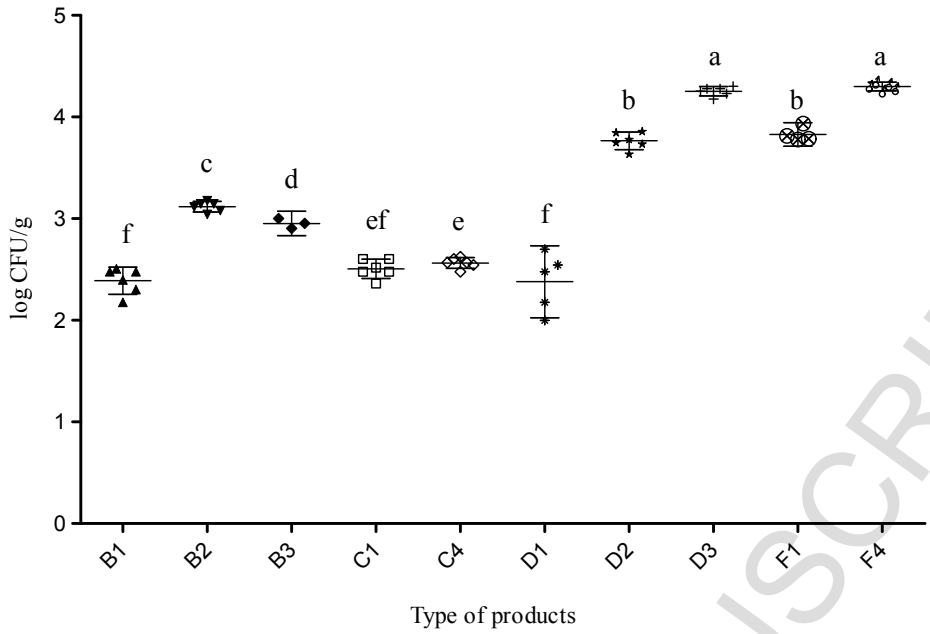


Fig.1. Quantification of coagulase-positive staphylococci in raw chicken meat products types

B , C and D represent exposed on ice, refrigerated with tray packing, and refrigerated without packing products in supermarkets respectively, F represents room temperature stored without packing products in wet markets. Consecutive numbers 1, 2, 3 and 4 represent drumstick, wings, breast and whole carcass respectively. Different lower-case letters represent statistical significance among different products types($p < 0.05$).

Table 1 Primers used in this study

| Gene | Sequence (5′-3′) | Reference |
|---|---|---|
| *16S* | CCACCTTCCTCCGGTTTGTCACC | (Perillo, et al., 2012) |
| | AACTCTGTTATTAGGGAAGAA | |
| *coa* | ACCACAAGGTACTGAATCAACG | (Perillo, et al., 2012) |
| | TGCTTTCGATTGTTCGATGC | |
| *nuc* | TGAAGTCAAATAAATCGCTTGC | (Perillo, et al., 2012) |
| | CCCTTTTCCACTAATTCCTTATTGT | |
| *sea* | CCTTTGGAAACGGTTAAAACG | (Omoe, et al., 2002) |
| | TCTGAACCTTCCCATCAAAAAC | |
| *seb* | TGTATGGTGGTGTAACTGAGCA | This study |
| | CCCGTTTCATAAGGTGAGTTGT | |
| *sec* | CTCAAGAACTAGACATAAAAGCTAGG | (Perillo, et al., 2012) |
| | TCAAAATCGGATTAACATTATCC | |
| *sed* | GTGGTGAAATAGATAGGACTGC | (Perillo, et al., 2012) |
| | ATATGAAGGTGCTCTGTGG | |
| *see* | CTGGAGGCACACCAAATAAA | This study |
| | TCCGTGTAAATAATGCCTTGC | |
| *seg* | AAGTAGACATTTTTGGCGTTCC | (Omoe, et al., 2002) |
| | AGAACCATCAAACTCGTATAGC | |
| *seh* | CAACTGCTGATTTAGCTCAG | (Perillo, et al., 2012) |
| | GTCGAATGAGTAATCTCTAGG | |
| *sei* | CAACTCGAATTTTCAACAGGTACC | (Perillo, et al., 2012) |
| | CAGGCAGTCCATCTCCTG | |
| *sej* | TGCACCTCCTCTCTGCGCCT | This study |
| | AGTGCATTGTAACGCCCCCGT | |
| *sek* | TAGGTGTCTCTAATAATGCCA | (Perillo, et al., 2012) |
| | TAGATATTCGTTAGTAGCTG | |
| *sel* | GCTTTCTGGAAGACCGTATCCTGTG | (Perillo, et al., 2012) |
| | GGCGATGTAGGTCCAGGAAACCT | |
| *sem* | ATGCTGTAGATGTATATGGTCTAAG | (Li, Wu, Wang, & Meng, 2015) |
| | CGTCCTTATAAGATATTTCTACATC | |
| *sen* | ATGAGATTGTTCTACATAGCTGCAAT | (Li, et al., 2015) |
| | AACTCTGCTCCCACTGAAC | |
| *seo* | TGTAGTGTAAACAATGCATATGCAAATG | (Li, et al., 2015) |
| | TTATGTAAATAAATAAACATCAATATGATGTC | |
| *sep* | TTAGACAAACCTATTATCATAATGG | (Li, et al., 2015) |
| | TATTATCATGTAACGTTACACCGCC | |
| *seq* | AAGAGGTAACTGCTCAAG | (Li, et al., 2015) |
| | TTATTCAGTCTTCTCATATG | |
| *ser* | AAACCAGATCCAAGGCCTGGAG | (Li, et al., 2015) |
| | TCACATTTGTAGTCAGGTGAACTT | |
| *seu* | TAAAATAAATGGCTCTAAAATTGATGG | (Li, et al., 2015) |
| | ATCCGCTGAAAAATAGCATTGAT | |

| | | |
|---|---|---|
| *tst* | GCTTGCGACAACTGCTACAG | (Perillo, et al., 2012) |
| | TGGATCCGTCATTCATTGTTAT | |
| *pvl* | ATCATTAGGTAAAATGTCTGGACATGATCCA | (Li, et al., 2015) |
| | GCATCAAGTGTATTGGATAGCAAAAGC | |
| *bla* | ACTTCAACACCTGCTGCTTTC | (Li, et al., 2015) |
| | TGACCACTTTTATCAGCAACC | |
| *mecA* | TGGCTATCGTGTCACAATCG | (Vazquez-Sanchez, Lopez-Cabo, Saa-Ibusquiza, & Rodriguez-Herrera, 2012) |
| | CTGGAACTTGTTGAGCAGAG | |
| *spa* | TAA AGACGATCC TTCGGTGAGC | (Normanno, et al., 2015) |
| | CAGCAGTAGTGCCGTTTGCTT | |
| *agr Ⅰ* | ATGCACATGGTGCACATGC | (Li, et al., 2015) |
| | GTCACAAGTACTATAAGCTG CGAT | |
| *agr Ⅱ* | ATGCACATGGTGCACATGC | (Li, et al., 2015) |
| | TATTACTAATTGAAAAGTGCCATAGC | |
| *agrⅢ* | ATGCACATGGTGCACATGC | (Li, et al., 2015) |
| | GTAATGTAATAGCTTGTATAATAATACCCAG | |
| *agrⅣ* | ATGCACATGGTGCACATGC | (Li, et al., 2015) |
| | CGATAATGCCGTAATACCCG | |

Table 2 Prevalence of coagulase positive staphylococci (CPS) in raw chicken meat at retail level

| Type of products | Supermarket (288) | | | | Wet market (176) | |
|---|---|---|---|---|---|---|
| | Frozen with plastic bagging | Exposed on ice | Refrigerated with tray packing | Refrigerated without packing | Frozen without packing | RT stored without packing |
| Drumstick | 0/24 | 12/24 | 6/24 | 4/24 | 0/32 | 7/32 |
| Wings | 0/24 | 5/24 | 0/24 | 3/24 | N/A | 0/32 |
| Breast | N/A | 1/24 | 0/24 | N/A | 0/16 | 0/32 |
| Whole carcass | N/A | 0/16 | 2/16 | 4/16 | 0/16 | 9/16 |

Table 3 Genotype distribution of *S.aureus* associated with raw chicken meat product categories

| Genotype | source | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Refrigerated with tray packing | | | Exposed on ice | | Refrigerated without packing | | | RT stored without packing | | |
| | a | b | c | a | d | a | b | d | a | d | |
| CC | 1(5*), 1(12) | 5(5) | 1(5) | 3(12),2(7),1(5) | 1(5) | 3(5), 1(12) | 1(5), 1(12) | 2(5) | 4(5) | 4(5) | 23(5),6(12),2(7) |
| ST | 1(12), 1(1) | 4(1), 1(8) | 1(2315) | 3(12),2(7),1(1) | 1(8) | 3(1), 1(12) | 1(1), 1(12) | 1(1),1(5) | 3(1),1(5) | 2(1),2(2315) | 16(1),2(5),2(7),2(8),6(12),3(2315) |
| *spa* | 1(t213),1(t127) | 4(t127), 1(t9101) | 1(t1687) | 3(t213), 2(t091), 1(t127) | 1(t9101) | 3(t127), 1(t213) | 1(t213), 1(t127) | 1(t213),1(t002) | 3(t127),1(t002) | 2(t127),2(t11687) | 16(t127),2(t002),2(t091),2(8),6(t213),3(t1687) |
| *blaZ* | 2 | 4 | 1 | 6 | 1 | 4 | 2 | 1 | 3 | 3 | 27 |
| *agr Ⅰ* | | 1 | | 2 | 1 | | | | | | 4 |
| *agr Ⅱ* | 1 | | 1 | 3 | | 1 | 1 | 1 | 1 | 2 | 11 |
| *agr Ⅲ* | 1 | 4 | | 1 | | 3 | 1 | 1 | 3 | 2 | 16 |
| *agr Ⅳ* | | | | | | | | | | | |
| *seb* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *sec* | 1 | | 1 | 4 | | 1 | 1 | | | 2 | 10 |
| *sed* | | 1 | | | 1 | | | | | | 2 |
| *see* | | 1 | | | 1 | | | | | | 2 |
| *seg* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *seh* | 1 | 4 | | 1 | | 3 | 1 | 1 | 3 | 2 | 16 |
| *sei* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *sej* | | 1 | | | 1 | | | | | | 2 |
| *sel* | 1 | | 1 | 4 | | 1 | 1 | | | 2 | 10 |
| *sem* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *sen* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *seo* | | | 1 | | | | | 1 | 1 | 2 | 5 |
| *sep* | | | | 2 | | | | | | | 2 |
| *ser* | | 1 | | | 1 | | | | | | 2 |

a represents drumstick, b represents wings, c represents breast, d represents whole carcass. * the number in parentheses represents genotype of CC, ST or *spa*.

Table 4 Antimicrobial resistance profiles of *S.aureus* isolated from raw chicken meat categories

| Source | | | Antimicrobial resistance profiles | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | TC | P | C | CM | K | CIP | E | S |
| Supermarket | Refrigerated with tray packing | Drumstick | 3 | 2 | | | | 1 | 1 | |
| | | Wings | 2 | 4 | | | | | 1 | |
| | | Breast | 2 | 1 | 1 | | | 2 | 1 | |
| | Exposed on ice | Drumstick | 5 | 6 | 2 | | | | 1 | 1 |
| | | Whole carcass | 1 | 1 | | | | 1 | 2 | |
| | Refrigerated without packing | Drumstick | 2 | 4 | | | | | | 1 |
| | | Wings | 2 | 2 | | | | 1 | 1 | 1 |
| | | Whole carcass | 3 | 1 | | | | 1 | 1 | |
| Wet market | Room temperature without packing | Drumstick | 3 | 3 | | 1 | | 2 | 2 | |
| | | Whole carcass | 3 | 3 | | | 1 | 3 | | |
| | Total (31 isolates) | | 26 | 27 | 3 | 1 | 1 | 11 | 10 | 3 |

TC-tetracycline, P-penicillin, C-chloromycetin, CM-clindamycin, K-kanamycin, CIP-ciprofloxacin, E-erythromycin, S-streptomycin.

Table 5 *S.aureus* isolates with strong biofilm formation ability (BFA) cultured in *vitro* related to raw chicken meat product categories

| Source | | | Strain | BFA* |
|---|---|---|---|---|
| Supermarket | Refrigerated without packing | Drumstick | Sa01 | 2.372±0.03 |
| | | Whole carcass | Sa02 | 8.741±0.06 |
| | Exposed on ice | Drumstick | Sa03 | 2.380±0.09 |
| | | Whole carcass | Sa04 | 2.247±0.08 |
| Wet market | Room temperature without packing | Drumstick | Sa05 | 10.313±0.00 |
| | | | Sa06 | 4.756±0.05 |

* Data shown as BFA mean±standard deviation.

**Reference**

Li, G., Wu, C., Wang, X., & Meng, J. (2015). Prevalence and characterization of methicillin susceptible Staphylococcus aureus ST398 isolates from retail foods. *Int J Food Microbiol, 196*, 94-97.

Normanno, G., Dambrosio, A., Lorusso, V., Samoilis, G., Di Taranto, P., & Parisi, A. (2015). Methicillin-resistant Staphylococcus aureus (MRSA) in slaughtered pigs and abattoir workers in Italy. *Food Microbiol, 51*, 51-56.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D. L., Ueda, S., & Shinagawa, K. (2002). Detection of seg, seh, and sei genes in Staphylococcus aureus Isolates and Determination of the Enterotoxin Productivities of S. aureus Isolates Harboring seg, seh, or sei Genes. *J Clin Microbiol, 40*(3), 857-862.

Perillo, J., Ceccarelli, D., Spagnoletti, M., Lollai, S., Cappuccinelli, P., & Colombo, M. M. (2012). Molecular characterization of enterotoxigenic and borderline oxacillin resistant Staphylococcus strains from ovine milk. *Food Microbiol, 32*(2), 265-273.

Vazquez-Sanchez, D., Lopez-Cabo, M., Saa-Ibusquiza, P., & Rodriguez-Herrera, J. J. (2012). Incidence and characterization of Staphylococcus aureus in fishery products marketed in Galicia (Northwest Spain). *Int J Food Microbiol, 157*(2), 286-296.