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***Running Head of the paper*:** genotypic characterization of *Staphylococcus aureus*

Enterotoxin genes and antimicrobial resistance in *Staphylococcus aureus* isolated from food products in Algeria

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Abstract

Aims : The aim of this study was to characterize *S. aureus* isolates of food origin (dairy and meat products, pastries, and sandwiches) determining the carriage in enterotoxin genes and the antimicrobial resistance pheno/genotypes.

Methods and results : A total of 300 food samples were collected and analyzed for the detection of *S. aureus*. The presence of enterotoxin genes was investigated by multiplex-PCRs. Resistance of isolates to eleven antimicrobials was determined using disc diffusion method and molecular characterization of methicillin-resistant *S. aureus* was carried out by *spa*-typing and MLST. Overall, 51 out of 300 samples (17%) were contaminated with *S. aureus*, and 104 isolates were recovered. Sixty-five of these isolates (62.5%) harbored one or more genes encoding for staphylococcal enterotoxins, being *seg* and *sei* the most observed genes. The highest resistance profile was ascribed to penicillin G (95.19%). Five isolates were methicillin-resistant (MRSA) harbouring the *mecA* gene. All MRSA isolates belonged to the sequence-type ST5 and to two different *spa*-types (t450 and t688); the MRSA-t450 isolate carried the *scn* gene (specific marker of the immune evasion cluster system), but the four MRSA-t688 isolates were *scn*-negative. The MRSA isolates carried enterotoxin genes but were negative for the genes of the Panton Valentine leukocidine (*lukF/S*-PV).

Conclusion : The presence of enterotoxigenic *S. aureus* isolates, including MRSA, in food samples can represent a risk for publich health.

Significance and impact of this study : This work describe the molecular characteristics of MRSA strains isolated from foods in Algeria and it can contribute to an extended database concerning the *S. aureus* isolated from food origin.

Key words : food products, *S. aureus*, enterotoxin genes, methicillin-resistant *S. aureus*, antimicrobial sensitivity.

Introduction

Food and food production may be a vehicle of antibiotic resistant bacteria and antibiotic resistance dissemination, which can be transmitted through the consumption of food animal products, including unpasteurized milk, meat or fish products (EFSA 2008; Ruiz and Alvarez-Ordóňez 2017). Many zoonotic organisms that are frequently resistant to antimicrobials and common causes of foodborne ilness are highly prevalent on farms, including nontyphoidal *Salmonella enterica*, *Campylobacter coli*, *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus* (Gebreyes *et al*. 2017).

Staphylococcus aureus is an important opportunistic pathogen for humans and animals (Lowy 1998). It is the causative agent of a variety of diseases ranging in severity from slight skin infections to more severe diseases, such as pneumonia, endocarditis, osteomyelitis, septicemia, or toxic shock syndrome, among others (Lowy 1998). In addition to staphylococcal infections, *S. aureus* is also responsible for food poisoning due to oral intake of enterotoxins present in foods (Pereira *et al*. 2009; Johler *et al*. 2015). Five enterotoxins (SEA, SEB, SEC, SED and SEE) are the most frequent ones associated with the staphylococcal food poisoning (SFPs) (Argudín *et al*. 2010). The food mostly involved in SFPs are milk and cream, cream filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings (Le Loir *et al*. 2003; Hennekinne 2018).

S. aureus can acquire antibiotic resistance determinants and therefore *S. aureus* isolates often exhibit resistance to multiple classes of antimicrobial agents (Papadopoulous *et al*. 2018). Methicillin-resistant *S. aureus* (MRSA) are of concern given that they represent a significant cause of morbidity and mortality throughout the world with important economic costs (Antonanzas *et al*., 2015; Castro et al., 2016). Methicillin resistance is conferred by the mobile genetic element, named the staphylococcal chromosomal cassette (SCC*mec*), carrying *mecA* or

mecC genes, encoding for production of an altered penicillin binding protein (PBP2a), with a low affinity for most of beta-lactam antimicrobials (Petinaki and Spiliopoulou 2012).

MRSA has been considered a major hospital associated pathogen (HA-MRSA), and has become a serious threat in hospitals worldwide (Oniciuc *et al*. 2017). However, MRSA has been also found associated to community setting (CA-MRSA) and to livestock (LA-MRSA) (Voss *et al*. 2005; Pantosti 2012). Recently, MRSA have been isolated in various types of food products, including raw milk, dairy products (Carfora *et al*. 2015; Caruso *et al.* 2016; Parisi *et al*. 2016; Basanisi *et al*. 2017; Giaciniti *et al*. 2017; Papadopoulos *et al*. 2019a; Titouche *et al*. 2019) and meat (Tang *et al*. 2017; Thapaliya *et al*. 2017). Transmission of zoonotic MRSA to humans can occur via either animal contact or contaminated food (Oniciuc *et al*. 2017). However, information about the potential transmission of MRSA to humans through the food chain remains limited (Petinaki and Spiliopoulou 2012). For this, continuous surveillance of MRSA along the food chain is essential to understand its role in the emergence and spread of antimicrobial-resistant pathogenic microorganisms.

In Algeria, the proportion of MRSA demonstrated a high increase in the community and healthcare settings; the European clone ST80-IV, producer of the Panton-Valentine Leukocidin (PVL), has being detected (Antri *et al*. 2011; Djahmi *et al*. 2013; Chaalal *et al*. 2018). A previous study of our group (Titouche *et al*. 2019) was focusing on analyzing dairy products obtained in 2014-2015, in which MRSA was isolated from some raw milk and acidified milk samples. In the present work, the objectives were : (1) to determine the prevalence of *S. aureus* in food products obtained during 2017-2018 (dairy and meat products, among others), (2) to evaluate the content in enterotoxin genes, (3) to characterize the MRSA isolates by *spa*-typing, MLST and antimicrobial resistance susceptibility testing.

MATERIALS AND METHODS

Sample Collection

A total of 300 samples of various food products including raw milk (54), dairy products (48), pastries (85), minced meat (85) and sandwiches (35) were collected during two years (2017 and 2018) from several randomly selected market points (butchers, cafeteria, creameries) located at Tizi Ouzou area (Algeria). All samples were collected aseptically in sterile bags, transferred immediately to the laboratory with ice packs and analyzed within 1 to 2 hours after sampling.

S. aureus isolation and biochemical identification

Ten mL (raw milk) or 10 g (minced meat, dairy products, sandwiches and pastries) of each sample was added to 90 mL of buffered peptone water (Conda Pronadisa, Madrid, Spain) and homogenized. Isolation of *S. aureus* was done by spreading 0.1ml of each resulting suspension on Baird-Parker base (Conda Pronadisa, Madrid, Spain) supplemented with 5% egg yolk and tellurite (Conda Pronadisa, Madrid, Spain). The plates were then incubated for 24-48h at 37°C. From each positive sample, one to five colonies (depending on their macroscopic structure and their charge in agar plates) with the typical aspect of *S. aureus* colonies (colonies with black appearance and surrounded by clear zone) were sub-cultured onto brain heart infusion (BHI) agar (Biokar, Beauvais, France) in order to obtain pure cultures. These were submitted to Gram staining to confirm coccus morphology. The isolates were identified using the conventional tests (catalase test, coagulase test, DNase ("thermonuclease") test and the Voges-Proskauer (VP) test for acetoïn production). The *S. aureus* ATCC25923 was used in microbiological analysis as a positive control. After identification, all isolates were stored in BHI broth (Biokar, Beauvais, France) with glycerol (30% V/V) at -20°C for further analysis.

Molecular characterization of S. *aureus* isolates

DNA extraction

Strains were grown overnight in BHI broth at 37°C and streaked onto a milk plate count agar (Bio Rad, Marnes la Coquette, France) incubated at 37°C for 24 h, prior to DNA extraction. DNA extraction was performed using the InstaGene Kit (Bio Rad, Marnes la Coquette, France) according to the manufacturer's recommendations. DNA concentrations were adjusted to 100 ng/µL using a Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington, USA).

Identification of *S. aureus* isolates by PCR amplification of 23S rRNA gene

PCR simplex was performed to detect the presence of 23S rRNA gene region specific for *S. aureus* according to Straub *et al*. (1999). The 1250 bp-long PCR products were electrophoresed in a 2% agarose gel and visualized by ethidium bromide (1µg/mL) staining using the Gel Doc EQ apparatus (Bio-Rad, Marnes la Coquette, France). DNA ladder 1kb (Promega, Lyon, France) was used as a molecular weight standard. The reference strain FRI361 was used as a positive control.

Detection of enterotoxin genes by multiplex PCR

Two multiplex PCR (mPCR) assays were used to detect *se* genes as described by Roussel *et al*. (2015). The first reaction (mPCR1) was performed with six primer pairs and allowed the detection of *sea*, *seb*, *sec*, *sed*, *see* and *ser* genes. The second reaction (mPCR2) was performed with five primer pairs and allowed the detection of *seg*, *seh*, *sei*, *sej* and *sep* genes. PCR amplification and electrophoresis of PCR products were performed as described previously (Roussel *et al*. 2015) according to the method developed and validated by the European Reference for coagulase positive staphylococci (EURL CPS). Five reference *S. aureus* strains (i.e, FRIS6, 374F, FRI137, FRI326 and FRI361) were used as positive controls.

148 **Detection of the *mecA*, *mecC*, *spa* and *lukS/F*-PV genes**

149 A multiplex PCR was performed as described by Stegger *et al*. (2012) in order to detect *mecA*
150 (162pb), *mecC* (138pb), Panton Valentine toxin (*lukF/S*-PV) (85pb) and *spa* (200-600pb)
151 genes, with modifications. DNA ladder 1kb (Promega, Lyon, France) was used as a molecular
152 weight standard. Three references strains were used as positive controls: *S. aureus* LGA251
153 (carrying *mecC* gene), *S. aureus* ATCC 25923 (carrying *pvl* gene) and *S. aureus* MU50
154 (carrying *mecA* gene).

155 **Characterization of MRSA strains by *spa*-typing and MLST**

156 The polymorphic x region of the *spa* gene was amplified by PCR with primers 1095F (5-
157 AGACGATCCTTCGGTGAGC-3) and 1517R (5-GCTTTTGCAATGTCATTTACTG-3)
158 (Harmsen *et al*. 2003). The PCR products were electrophoresed in a 1% agarose gel and
159 visualized using the Gel Doc EQ apparatus (Bio-Rad, Marnes la Coquette, France). They were
160 further sequenced by Genewiz, on both DNA strands. *spa*-types were determined from the
161 resulting DNA sequences using the Ridom® Staph-type software.

162 Multilocus sequence typing (MLST) was performed in MRSA strains as previously described
163 (Lozano *et al*. 2012): the allelic profile of each isolate was obtained by sequencing internal
164 fragments of seven housekeeping genes (*arcC, aroE, glpF, gmK, pta, tpi* and *yqiL*), allowing
165 the determination of the sequence type (ST), by the MLST database (http://saureus.mlst.net/)

166 **Antimicrobial susceptibility of *S. aureus* isolates**

167 Antimicrobial susceptibility was determined by the disc diffusion method on Mueller-Hinton
168 agar (Biokar, Beauvais, France) according to the guidelines of the Clinical and Laboratory
169 Standards (CLSI, 2018). The antibiotics discs from Liofilchem (Roeseto, Italy) are listed as
170 follows (antibiotic concentration in µg): penicillin G (10 UI), cefoxitin (30), gentamicin (10),
171 tobramycin (10), neomycin (30), tetracycline (30), erythromycin (15), ofloxacin (15),

clindamycin (2), chloramphenicol (30), and trimethoprim/sulfamethoxazole (1.25/23.75). The strains were classified as susceptible, intermediate or resistant according to breakpoints of the clinical and laboratory standards (CLSI, 2018). Control strain *S. aureus* ATCC 25923 was used in susceptibility testing. Phenotypic detection of MRSA was performed by cefoxitin-disk-diffusion test.

Detection of antimicrobial resistance genes and the *scn* gene of the immune-evasion-cluster (IEC) in MRSA strains

The presence of several genes that confer resistance to penicillin (*blaZ*), tetracycline (*tet*K, *tet*M and *tet*L), macrolides/lincosamides (*erm*A, *erm*B, *erm*C, *msr*A and *mph*C), phenicols (cat_{pC221}, cat_{pC223}, cat_{pC194}, *fex*A, *fex*B, *cfr*), and aminoglycosides (*ant*[4′]-*Ia*) was analyzed by PCR, as described previously (Lozano *et al*. 2012; Ruiz-Ripa *et al*. 2019).

All MRSA strains were tested by PCR for the presence of the *scn* gene, marker of the Immune Evasion Cluster (IEC), as previously reported (Lozano *et al*. 2012).

Results

Prevalence of S. aureus

Out of 300 food products samples, 51 (17%) samples showed *S. aureus* contamination, including the following samples: 20 (37.04%) raw milk, 12 (14.46%) pastries, 6 (7.05%) minced meat, 1 (4.35%) butter, 6 (100%) rayeb and 6 (50%) l'ben. No contamination with *S. aureus* was observed for sandwichs and yougurt (Table 1).

A total of 104 isolates were obtained from the 51 positive samples. Most of them were isolated from pastries (39) and raw milk (33). The remaining isolates were retrieved from minced meat (6), butter (4), rayeb (6) and l'ben (16) (Table 3).

Occurrence of enterotoxin genes

Of the 104 recovered isolates, 65 (62.5%) carried at least one gene encoding for staphylococcal enterotoxins. Twenty four (36.92%) of these isolates possessed one kind of

enterotoxin genes, and the remaining (63.07%) harbored more than one. Ten staphylococcal genotypes were observed, the most detected were *sei* and *seg* (47.69%), followed by *seb* (23.08%). Other genotypic profiles were detected with low frequencies (Table 2). None of the isolates contained *sed* or *see* genes.

Antimicrobial resistance of S. aureus isolates

Out of 104 *S. aureus* isolates tested, 99 (95.19%) were found to be resistant to at least one antimicrobial and 16 (15.38%) were multi-drug resistant. The highest resistance rate was observed for penicillin G (95.19%) (Table 3). All isolates were susceptible to sulfamethoxazole/trimethoprim and ofloxacin. However, low resistances were observed against neomycin (23.08%), tetracycline (17.31%), tobramycin (6.73%), erythromycin (5.77%), cefoxitin (4.81%), chloramphenicol (4.81%), gentamicin (0.96%) and clindamycin (0.96%). Six phenotypes of multidrug resistance were observed (Table 4).

Detection and characterization of MRSA isolates

Five MRSA isolates (4.81%) were identified (resistant to cefoxitin), and harbored the *mecA* gene. Four isolates were recovered from rayeb and one from raw milk (Table 5). None of them carried the Panton Valentine leukocidine toxin (*luk*F/*S*-PV) genes. Typing of the MRSA isolates indicated that they belonged to the same sequence type ST5, but to two different *spa*-types : t450 and t688. Four MRSA isolates carried the *bla*Z, *tet*K, *tet*M and *tet*L genes (encoding for penicillin and tetracycline resistance, respectively), *fex*A (chloramphenicol resistance), and *erm*B and *erm*C (erythromycin resistance) and the remaining isolate carried *blaZ*, *tet*K, *tet*L, *tet*M, *ant*4 (tobramycin resistance), and *erm*C genes (erythromycin resistance) (Table 5). All these isolates harbored enterotoxin genes. Only, one MRSA strain harbored the *scn* gene of the IEC system.

Discussion

In our study, the *S. aureus* contamination rate was lower than previously observed by Chaalal *et al*. (2018) in Algeria. These authors reported a prevalence of 30.9% out of 495 analyzed samples, including raw and processed foods. However, the prevalence obtained in this study was higher than that previously observed by Mairi *et al*. (2019), with rate of 8.6%. A higher prevalances of *S. aureus* have been observed in other countries concerning various foodstuffs, including raw milk and dairy products (Rola *et al*. 2015; Mehli *et al*. 2016; Obaidat *et al*. 2018; Ahmed Abdel-Hameid *et al*. 2019; Papadopoulous *et al*. 2019b), meat and meat products (Tang *et al*. 2017; Wu *et al*. 2018) and ready to eat products (Islam *et al*. 2019). The differences between results obtained from various studies about prevalence rates of *S. aureus* may be related to different attributes, including sources of samples, geographical origin, innappropriate antimicrobial administrations, sensitivity of the identification methods, sample size, storage and handling of samples (Al-Ashmawy *et al*. 2016; Gharsa *et al*. 2018). Various sources of food contamination by *S. aureus* were described, including poor hygienic practices during production, processing, cooking and distribution (Jamali *et al*. 2015; Hennekinne 2018). However, food handlers can constitute the main source of food contamination, via carrying of enterotoxin-producing *S. aureus* in their noses or on their hands (Tan *et al*. 2014; Castro *et al*. 2016).

The pathogenicity of *S. aureus* is related to various virulence factors. Heat stable staphylococcal enterotoxins (SEs) produced by enterotoxigenic strains of *S. aureus* is considered as one major global cause of food poisoning (Le Loir *et al*. 2003). A high prevalence of enterotoxigenic *S. aureus* was observed in our study. Our results corroborate with those announced by Khemiri *et al*. (2019), who repported that 87.5% of isolates from raw milk were positive for one or more staphylococcal enterotoxins. The same prevalences were observed in strains isolated from various foods, including raw milk, dairy products, meat and ready to eat

products in other countries (Normano *et al*. 2007a; Pereira *et al*. 2009; Aydin *et al*. 2011; Carfora *et al*. 2015; Mehli *et al*. 2017). Although *sec* and *sed* are the most reported enterotoxin genes in foods, *sei* and *seg* were the most prevalent enterotoxin genes in our study. SEA, SEB and SED, either alone or together with other staphylococcal enterotoxins, are the most commonly reported in foods, and are also the main cause of staphylococcal food poisonning (SFP) (Argudin *et al*. 2010).

The high level of penicillin resistance observed in this study is consistent with many other authors (Pereira *et al*. 2009; Jamali *et al*. 2015; Chaalal *et al*. 2018; Obaidat *et al*. 2018; Gharsa *et al*. 2019; Papadopoulos *et al*. 2019b). The emergence and spread of antimicrobial resistance has been usually attributed to the misuse or indiscriminate use of antibiotics as therapeutic drugs in human and animal health care or as growth promoters in veterinary husbandry (Ruiz and Alvarez-Ordóñez 2017). All isolates were susceptible to sulfamethoxazole/trimethoprim and ofloxacine. The same results were obtained in previous studies (Jamali *et al*. 2015; Achek *et al*. 2018). A slight resistance was observed to chloramphenicol, which suggests its use in veterinary medecine. These results corroborate with those observed by Jamali *et al*. (2015) in strains isolated from cow milk and sheep milk, respectively. A low resistances for neomycin, tetracycline, erythromycin and clindamycin were detected. These results agree with those reported by previous studies (Pereira *et al*. 2009; Jamali *et al*. 2015; Achek *et al*. 2018; Mairi *et al*. 2019).

In the present work, a low prevalence of MRSA was observed. In Algeria, little data are available concerning MRSA in foods. Chaalal *et al*. (2018) reported a rate of 21.5% in various type of foods, including raw milk, meat and pastry. A low prevalences were observed in other studies, with a values of 4.1% in raw milk and traitional dairy products (Titouche *et al*. 2019) and 13.6% (Mairi *et al*. 2019). All MRSA strains of our study were isolated from raw milk and dairy products (rayeb). As reported by many authors, raw milk has been identified as a source

of MRSA demonstrating the potential food safety of contaminated milk and dairy products entering the human food chain (Carfora *et al*. 2016; Caruso *et al*. 2016; Parisi *et al*. 2016; Basanisi *et al*. 2017; Giaciniti *et al*. 2017; Papadopoulos *et al*. 2019b; Titouche *et al*. 2019). However, MRSA were also isolated in meat (De Boer *et al*. 2009; Hanson *et al*. 2011; Thapaliya *et al*. 2017; Tang *et al*. 2017) and from ready to eat products (Wang *et al*. 2017; Islam *et al*. 2019). To date, a great number of studies reported the isolation of MRSA from livestock, wild animals and derived foods, both raw and ready to eat, as well as from professionals working in animal husbandry or the food production chain settings (Sergelidis and Angelidis 2017). Transmission of zoonotic MRSA to humans can occur via either animal contact or contaminated food (Oniciuc *et al*. 2017).

The antimicrobial susceptibility of MRSA strains revealed that all of these strains were resistant to antimicrobial agent other than β-lactams, such as tetracycline, aminoglycosides (tobramycin and neomycin), macrolides (erythromycin) and chloramphenicol, indicating a multidrug-resistant phenotype, as in other studies (Caruso *et al*. 2016; Parisi *et al*. 2016; Chaalal *et al*. 2018; Papadopoulos *et al*. 2019b; Titouche *et al*. 2019). None of our MRSA strains carried the genes encoding the Panton Valentine leucocidin (*lukF/S*-PV), although these genes have been detected by others in raw milk samples (Haran *et al*. 2012; Basanisi *et al*. 2017; Chaalal *et al*. 2018), meat (Hanson *et al*. 2011; Thapaliya *et al*. 2017; Chaalal *et al*. 2018) and ready to eat foods (Chaalal *et al*. 2018; Islam *et al*. 2019).

Similar to our results, previous studies have reported the ability of MRSA strains to carry staphylococcal enterotoxin genes (Normano *et al*. 2007b; Haran *et al*. 2012; Parisi *et al*. 2016; Rodríguez-Lázaro *et al*. 2017; Papadopoulos *et al*. 2019b; Titouche *et al*. 2019). However, in contrast to data on *Salmonella* spp, *Campylobacter* spp, and *Shigella* spp, which show clearly the involvement of antimicrobial-resistant strains in food-borne outbreaks, there are only a few studies on the occurrence of MRSA strains in staphylococcal food poisoning (Sergelidis and

297 Angelidis 2017). Jones *et al*. (2002) described the first report of an outbreak of gastrointestinal
298 illness caused by community acquired MRSA, and Kerouanton *et al*. (2007) identified two
299 MRSA out of 33 *S. aureus* strains recovered from staphylococcal food poisoning. Although,
300 transmission of MRSA strains from farm animals to humans has been well documented,
301 informations about the potential transmission of MRSA to humans through the food chain
302 remains limited (Petinaki and Spiliopoulou 2012).

303 With regard to genetic typing, all MRSA strains isolated in this study belonged to the same
304 sequence type ST5 and to two *spa*-types : t450 and t688. It is of interest to remark that the four
305 t688 strains were *scn*-negative (marker of IEC system) and the strain t450 was the *scn*-positive,
306 suggesting an animal and human origin, respectively. Our results are in line with those of Parisi
307 *et al*. (2016) and Basanisi *et al*. (2017), who isolated MRSA strains ST5 (t688) from bulk tank
308 milk and cheese samples in Italy. The ST5 lineage can be considered an animal-adapted clone,
309 since it reported in humans as well as companions animals, poultry, pigs and cattle (Pantosti
310 2012). Since the emergence of this MRSA clone associated with livestock, molecular typing
311 methods have confirmed the relationship of this strains with food production animals, and
312 humans in contact with these animals. From these reservoirs, MRSA can be introduced into
313 hospitals, causing serious infections and outbreaks (Lakhundi and Zhang 2018).

314 The high presence of staphylococcal enterotoxin genes in our isolates, as well as the detection
315 of MRSA strains is of concern and constitutes a public health hazard, because these strains can
316 be disseminated through the community, causing food poisoning. Our results indicate the need
317 for continuous monitoring and improvement of hygienic quality of food products, by ensuring
318 of proper conditions of handling and production.

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Conflict of Interest

Authors declare no conflict of interest.

Authors contributions

Yacine Titouche : performed the experiments, analyzed the data and wrote the manuscript. Jacques Antoinne Hennekinne, Carmen Torres, Karim Houali, Yacine Nia, Abdelhak Fatihi and Alexandra Cauquil : supervised the experiments, analyzed the results and revised the different version of the manuscript. Laura Ruiz-Ripa : performed part of experiment and revised the manuscript. Noémie VINGADASSALON, Pascal BOUCHEZ and Laurence BOUHIER : collaborated in the design of experiments

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Table 1 Occurrence of *S. aureus* in food products

Product	Number of analyzed samples	Number of positive samples (%)
Raw milk	54	20 (37.04)
Pastries	83	12 (14.46)
Minced meat	85	6 (7.05)
Butter	23	1 (4.35)
Rayeb	06	6 (100)
L'ben	12	6 (50)
Sandwichs	35	0 (0)
Yougurt	02	0 (0)
Total	300	51 (17)

Table 2 Ditribution of enteroxin gene profiles among the *S. aureus* isolates of the study

Origin	No of enterotoxigenic *S. aureus* isolates	No of enterotoxigenic *S. aureus* isolates according their isolation origin									
		sea	*seb*	*sec*	*seh*	*sep*	*seg* + *sei*	*sea* + *seh*	*sea* + *seg* + *sei*	*ser* + *seg* + *sei*	*seb* + *ser* + *seg* + *sei* + *sej*
Minced meat	02	---	1	----	----	-----	1	-----	-----	-----	------
Pastries	25	2	4	----	----	-----	18	------	------	1	------
Raw milk	24	----	2	4	1	2	10	4	1	----	----
Butter	02	---	2	---	----	---	----	----	-----	-----	-----
Rayeb	05	---	1	----	----	----	-----	-----	------	-----	4
L'ben	07	----	5	----	-----	-----	2	-----	------	------	-----
Total	65	2 (3.08%)	15 (23.08%)	4 (6.15%)	1 (1.54%)	2 (3.08%)	31 (47.69%)	4 (6.15%)	1 (1.54%)	1 (1.54%)	4 (1.54%)

Table 3 Number and percentage of *S. aureus* isolates resistant to different antimicrobials

Origin	No of *S. aureus* isolates	No of *S. aureus* isolates (**%**) resistant to antimicrobial according their isolation origin										
		P	FOX	TE	CN	N	TOB	E	C	SXT	OFX	CD
Minced meat	6	5 (83.33)	0 (0)	0 (0)	0 (0)	3 (50)	1 (16.67)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pastries	39	39 (100)	0 (0)	1 (2.56)	0 (0)	6 (15.38)	1 (2.56)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Raw milk	33	31 (93.94)	1 (3.03)	3 (9.09)	1 (3.03)	4 (12.12)	2 (6.06)	1 (3.03)	0 (0)	0 (0)	0 (0)	0 (0)
Butter	4	4 (100)	0 (0)	3 (75)	0 (0)	4 (100)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	1 (25)
Rayeb	6	6 (100)	4 (66.67)	5 (83.33)	0 (0)	1 (16.67)	0 (0)	4 (66.67)	4 (66.67)	0 (0)	0 (0)	0 (0)
L'ben	16	14 (87.56)	0 (0)	6 (37.5)	0 (0)	6 (37.5)	3 (18.75)	0 (0)	1 (6.25)	0 (0)	0 (0)	0 (0)
Total	104	99 (95.19)	5 (4.81)	18 (17.31)	1 (0.96)	24 (23.08)	7 (6.73)	6 (5.77)	5 (4.81)	0 (0)	0 (0)	1 (0.96)

P : penicillin, FOX : cefoxitin, TE: tetracycline, CN : gentamicin, N: neomycin, TOB: tobramycin, E: erythromycin, C : chloramphenicol, SXT : sulfamethoxazole/trimethoprim, OFX: ofloxacin, CD: clindamycin

Table 4 Phenotypic resistance patterns among multidrug-resistant *S. aureus* isolates

Antimicrobial resistance phenotye	Number of isolates
P-N-TE	6
P-TOB-N-C	1
P-N-E-CD-TE	1
P-TOB-N-TE	3
P-FOX-E-C-TE	4
P-FOX-TOB-N-E-TE	1
Total	16

P : penicillin, FOX : cefoxitin, TE: tetracycline, CN : gentamicin, N: neomycin, TOB: tobramycin, E: erythromycin, C : chloramphenicol, CD: clindamycin

Table 5 Phenotypic and genotypic characterization of MRSA isolates

Strain	Origin	*spa*-type	ST	Toxin gene profile	Phenotype of resistance	*mecA*/*mecC*	*pvl*	*scn*	Antimicrobial resistance genes
S201	Raw milk	t450	ST5	*seg* ; *sei*	P-FOX-E-TOB- N-TE	*mecA*	-	+	*blaZ*, *mecA*, *ant*4, *erm*C, *tet*(K), *tet*(M), *tet*(L)
S246	Rayeb	t688	ST5	*seb ; ser* ; *seg* ; *sei* ; *sej*	P-FOX-E-C-TE	*mecA*	-	-	*blaZ*, *mecA*, *erm*C, *erm*B, *fex*A, *tet*(M), *tet*(K)
S247	Rayeb	t688	ST5	*seb ; ser* ; *seg* ; *sei* ; *sej*	P-FOX-E-C-TE	*mecA*	-	-	*blaZ*, *mecA*, *erm*C, *erm*B, *fex*A, *tet*(M), *tet*(K)
S249	Rayeb	t688	ST5	*seb ; ser* ; *seg* ; *sei* ; *sej*	P-FOX-E-C-TE	*mecA*	-	-	*blaZ*, *mecA*, *erm*C, *erm*B, *fex*A, *tet*(M), *tet*(K)
S250	Rayeb	t688	ST5	*seb ; ser* ; *seg* ; *sei* ; *sej*	P-FOX-E-C-TE	*mecA*	-	-	*blaZ*, *mecA*, *erm*C, *erm*B, *fex*A, *tet*(M), *tet*(K)

ST : sequence type