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Daniela Bencardino, Luca Agostino Vitali

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Title

Staphylococcus aureus carriage among food handlers in a pasta company: pattern of virulence and resistance to linezolid.

Author names and affiliations

Daniela Bencardino[a,b,], Luca Agostino Vitali[b]

[a]School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino, Italy

[b]Microbiology Unit, School of Pharmacy, University of Camerino, Via Gentile III da Varano, 62032 Camerino, Italy.

E-mail addresses: daniela.bencardino@unicam.it (D. Bencardino), luca.vitali@unicam.it (L.A. Vitali)

Corresponding author:
Daniela Bencardino
School of Biosciences and Veterinary Medicine
University of Camerino
Via Gentile III da Varano
62032 Camerino (MC) – Italy
tel: 0039-0737-403286
Fax: 0039-0737-403290
E-mail: daniela.bencardino@unicam.it

Abstract

This study aimed at monitoring and characterize the *Staphylococcus aureus* carriage status of employees in a pasta company in order to evaluate the associated risk factors. Food handlers (n=21) were sampled between 2013 and 2015 through nasal and hand swabs to determine the colonization status. Seven out of 21 employees (33%) were contaminated with *S. aureus* and the prevalence decreased to 9.5% over the last year. Only two persistent carriers were identified. Twenty-eight strains were isolated from both hand and nasal samples. Each of them was resistant to at least one class of antibiotics and the multidrug resistance strains were isolated from the nose. The highest resistance rate was observed towards penicillin G (79%) and to linezolid (64%) confirming the rapid spread of linezolid resistant strains recently described in Italy. The dominant toxin gene was *sem* (93%), which is usually not among the most prevalent, whereas the primary *agr* group was the *agrIII* (43%) and the most frequent *spa* type was t030 (39%). These results combined with the genomic macrorestriction analysis revealed high genetic diversity. The increased virulence, antibiotic resistance and molecular variability of isolates highlighted the importance of monitoring activity in food company to assess the potential associated risk of foodborne diseases.

Keywords

Staphylococcus aureus; Food handlers; Carriage monitoring; Molecular typing; Food safety; Contamination

1. Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic human pathogen causing a variety of self-limiting to life-threatening diseases. The preferred colonization sites are the anterior part of the nares and hand surfaces and the 12 to 30% of healthy population is persistently colonized by this pathogen (Zanger, Nurjadi, Vath, & Kremsner, 2011). Because *S. aureus* is a common human commensal of the hand and mucosal membranes, the colonized and asymptomatic carrier handlers can contaminate food through manual contact or respiratory secretions. In fact, food contamination is largely associated with improper product manipulation and poor hygiene conditions (Argudín, Mendoza, & Rodicio, 2010). Thus, The spread of staphylococcal food poisoning (SFP) via food handlers is an important problem worldwide and it is caused by the ingestion of food contaminated by enterotoxigenic strains (Johler, Giannini, Jermini, Hummerjohann, Baumgartner, & Stephan, 2015). The 95% of SFP are caused by the classical staphylococcal enterotoxins (SEs) named as SEA, SEB, SEC, SED and SEE whereas the rest is caused by the newly identified (SEG-SEI-SEM-SEN and SEO). These virulence factors are highly stable to heat, to low pH and to proteolytic enzymes reducing the efficacy of thermic and acidification industrial procedures, usually applied to minimize the microbial hazard in the final product (Kadariya, Smith, & Thapaliya, 2014). Hence, the successful persistence of the pathogen in various food matrices increases the associated risk. The SFP symptoms, including nausea, vomiting and diarrhoea, can appear rapidly (from 2 to 8 hours). Typically, the disease resolves within 24–48 hours, but severe cases require hospitalization and the death occurs in the most susceptible people (Argudín et al., 2010). Many investigations have reported cases of *S. aureus* carriers among food handlers as potential source of food contamination (Hatakka, Björkroth, Asplund, Mäki-Petäys & Korkeala, 2000; Leibler, Jordan, Brownstein, Lander, Price & Perry, 2016; Castro, Santos, Meireles, Silva, & Teixeira, 2015). Furthermore, the food handlers have been recognized as an important reservoir of antimicrobial resistant strains and the contaminated foods can act as vehicles of transmission, especially those that are not heat-treated (Argudín, Mendoza, González-Hevia, Bances, Guerra, & Rodicio, 2012). The extraordinary capability and the mechanisms of *S. aureus* to develop antibiotic resistance are known (Foster, 2017). The emergence of virulent and multidrug resistant strains highlights the necessity to monitor the healthy status of food handlers to prevent the risk of SFP. The present study aimed at determining the occurrence of *S. aureus* healthy carriers among the handlers working in a pasta company. In order to evaluate the risk associated to the contamination of food products, the genotypic and phenotypic characterization of isolates was carried out. Besides the analysis of resistance and virulence profiles also the clonality of strains was investigated.

2. Materials and methods

2.1 Bacterial isolates

This investigation was carried out in a pasta company of Marche Region, Italy. The overall purpose was to identify the source of contamination found during the process resulting in a

final product positive to *S. aureus*. The monitoring of food carrier-handler status was a part of an implemented microbiological surveillance programme. The hand and the nasal swabs from 21 food handlers were collected between February 2013 and September 2015. Sampling was done every 4 months during the first year and every 6 months during the last two years. It was performed during the working hours and in the same day for all members of the staff. Each handler was indicated with a number from 1 to 21. Their job functions were related to the manual packaging of the final product and all worked in the same area. All the food handlers gave their informed consent for sampling as per the agreement to the company safety procedures. All workers approved the protocol. Nasal and hand samples were collected using sterile cotton swab as described by Hu, Umeda, Kondo, & Amako (1995) and by Tan, Lee, Abu Bakar, Abdul Karim, Rukayadi, & Mahyudin (2013), respectively. The collected swabs were streaked onto Baird-Parker plates and aerobically incubated at 37°C for 24 hours. The GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) was used for the extraction of the genomic DNA and the presumptive assignments to the species *S. aureus* were confirmed by PCR detection of the thermonuclease gene *nuc* (Brakstad, Aasbakk, & Maeland, 1992). The primers and the annealing temperature are reported in the supplementary material (table S1). The carrier-handlers were subjected to a treatment with the antibiotic mupirocin as described by the HACCP plan of the company.

2.2 Antibiotic susceptibility

The antibiotic susceptibility of all isolates was determined in accordance with the guidelines of the European committee on antimicrobial susceptibility testing (http://www.eucast.org; last date accessed: 27 June, 2018). The antibiotics tested by means of the disc diffusion method were (absolute amount of the respective molecule is indicated in parenthesis): penicillin (1 µg), oxacillin (1 µg), vancomycin (5 µg), cefoxitin (30 µg), tetracycline (30 µg), thrimethoprim-sulfamethoxazole (25 µg), fusidic acid (10 µg), clindamycin (2 µg), erythromycin (15 µg), levofloxacin (5 µg), linezolid (10 µg). The E-test was applied to assess susceptibility to daptomycin as per the same EUCAST guidelines (E-test strips were from Oxoid).

2.3 PCR screening of resistance genes

All resistant strains were investigated by PCR amplification to detect the associated resistance genes. The list of genes, primer pairs used and annealing temperatures applied are provided in table S1. Erythromycin-resistant isolates were screened for the presence of *ermA*, *ermB*, *ermC* and *msrA* genes. The *blaZ* gene was explored in penicillin resistant isolates, (Gómez-Sanz, Torres, Lozano, Fernandéz-Pérez, Aspiroz, Ruiz-Larrea, & Zarazaga, 2010). The *linA* gene was detected for the strains resistant to clindamycin but negative for the *ermA/B/C* genes that confer the resistance to macrolide, lincosamide, and streptogramin (MLS) antibiotics of type B (Lina, Quaglia, Reverdy, Leclercq, Vandenesch, & Etienne, 1999). The presence of acquired fusidic acid resistance determinants (*fusA, fusB, fusC, fusD*) was assessed by PCR and then sequencing of the obtained amplicons, as described by Chen et al., (2010). In the case of

linezolid resistant strains negative for the *cfr* gene, the presence of mutations in 23S rDNA genes and those in *rplC* and *rplD* genes were analysed as described by Bongiorno et al., (2010).

2.4 Detection of toxin genes

The selected toxin genes (*sea, sec, sei, sem, seo, tst*) were amplified by PCR using the primer sequences reported in Table S1. The reaction mix (25 µl/tube) contained 1 µg of chromosomal DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates (dNTPs), 1 µM oligonucleotide primer, and 0.5 U Taq polymerase (AmpliTaq Gold; Applied Biosystems). The thermal cycling conditions were 30 cycles at 94°C for 30 sec, 40 sec at primer specific annealing temperature (table S1), 72°C for 30 sec and one cycle at 72°C for 7 min. The PCR products were separated by electrophoresis on a 1.5% (w/v) agarose/ethidium bromide gel (Sigma-Aldrich) finally recorded using UV transillumination. A 1 kb DNA ladder was included in all fingerprinting gels as a molecular weight standard. The PCR products were of the expected lengths.

2.5 Molecular typing

The strain relatedness was analysed by pulsed-field gel electrophoresis (PFGE) of the SmaI macro-restricted genomic DNA according to the procedure described by Chung, De Lencastre, Matthews, & Tomasz (2000). Additionally, all isolates were typed to determine the *agr* group and the variants of the gene expressing the protein A. The *agr* group was determined by PCR amplification according to Jarraud et al., (2002). The staphylococcal protein A typing (*spa* typing) was carried out using primers *spa*-1113f and *spa*-1514r as described by Shopsin et al. (1999). Specific primers and annealing temperatures are reported in table S1. The PCR products were purified using the PCR Clean up kit (Sigma-Aldrich, St. Louis, MO) and sequenced. The sequences were analysed using Ridom SpaServer website (http://www.spaserver.ridom.de; last date accessed: 27 June, 2018) to determine the *spa* type.

3. Results

3.1 Carriage status

Of the 21 employees analysed, 7 (33%) were contaminated with *S. aureus*. Most of them were nasal carriers (78%). Different strains were isolated from the same worker. The prevalence of carriers was 33%, 19%, and 9.5% in 2013, 2014, and 2015, respectively. Only two handlers were persistent carriers (table 1). The persistent carriers, indicated as number 2 and 5, were colonized by three or more strains during the first and the second year. Similar strains were found in both nose and hand of handlers, but no hand colonization was recorded during the last year. Three handlers out of seven were positive for *S. aureus* both in the nares and the hand showing, in some cases, the same strain (table 2). None of them underwent an antibiotic treatment in the trimester preceding our sampling. The percentage of carriers decreased over the three years of the surveillance as a consequence of the antibiotic decolonization strategy.

3.2 Antibiotic resistance

A total of 28 *S. aureus* were collected from hand and anterior nares but no MRSA were present. Each isolate was resistant to at least one antimicrobial agent. Only five strains (18%) from the anterior nares were multidrug resistant (resistance to more than two antibiotics). The highest prevalence in resistance was that towards penicillin G (22 strains, 79%) always associated with the presence of *blaZ*. The fractions of strains resistant to linezolid and erythromycin were 68% and 32%, respectively. Only one strain was resistant to clindamycin and another one to fusidic acid. The strains resistant to erythromycin carried the *msrA* gene, while resistance to clindamycin was associated with the presence of *linA*. The single fusidic acid non-susceptible strains was positive to *fusB*. *cfr* was absent in linezolid resistant strains, which were further investigated for mutations in the genes coding for 23S rRNA and L3, L4 ribosomal proteins. Sequence analysis showed that no mutation associated to linezolid resistance were evident in L3 and L4, whereas there was a point mutation (G-to-T at position 2,576) in the 23S rDNA gene.

3.3 Distribution of toxin genes

The most prevalent toxin gene was *sem*, found in 26 (93%) strains. The two strains negative for *sem* were the only ones collected during 2015 and isolated from the nose of persistent carriers. Fifteen isolates (54%) were positive for *sec*, 14 (50%) for *tst*, 8 (29%) for *seo* and only one isolate carried the *sei* gene. One of the nasal strains isolated during 2013 was positive for 5 toxin genes. Overall, 11 different genotypes were discriminated according to the pattern of toxin genes (table 2). The most prevalent genotype (8 isolates-29%) was the sem^+ without marked incidence in nasal or hand samples. The $sea^+tst^+sem^+sec^+$ was characteristic of 7 isolates (25%) that were collected from the anterior nares of three handlers between 2013 and 2014 and were resistant to penicillin and linezolid.

3.4 *agr* and *spa* typing

Polymorphisms in the *agr* locus allow the classification of *S. aureus* strains into four different groups (Simpson's Index of Diversity: 0.675; C.I.95%: 0.619-0.730). The dominant *agr* group detected was the *agrIII* (43%), followed by the *agrII* (32%) and *agrI* (25%). No *agrIV* was recorded and the *agrIII* was frequently observed among the toxin genotypes with three or more genes. Sequence analysis of region X of the *spa* gene sorted 7 distinct *spa* types (t002, t012, t026, t030, t10307, t209, t1192). Its discriminatory power was very strong (Simpson's Index of Diversity: 0.751; C.I.95%: 0.647-0.856). The most frequent *spa* type was t030 (39%) and it was detected among all of the four PFGE types (table 2). The remaining *spa* types, with the exception of t026, were all associated with a single PFGE type. The *spa* types t030, t012 and t10307 shared similar repeats (table 3). Considering the limited number of collected strains (some clusters count less than five strains) and the high genetic variability detected, we are not able to describe statistical correlation between *spa* types and other molecular features. However, some considerations can originate from the general analysis of virulence and antibiotic patterns of the two prevalent *spa* types. Most nasal strains of t030 resulted related to sea^+,tst^+,sem^+,sec^+ pattern and resistant to penicillin and linezolid. This pattern was observed in strains isolated from two different handlers in the same year (2013). A large number of strains

typed as t026 were characterized by the presence of *sem*$^{+}$,*sec*$^{+}$,*tst*$^{+}$ toxin gene and resistant to linezolid. No particular differences in terms of *spa*, virulence and antibiotic patterns were detected during the three years.

3.5 PFGE typing

PFGE distinguished 4 different patterns among 28 strains. The patterns were arbitrarily indicated as A, B, C and D and differed one from each other for three or more bands. This high variability showed that the strains were not closely related (Simpson's Index of Diversity: 0.712; C.I.95%: 0.605-0.819). The pattern A included 13 strains: 8 (62%) were from the nose and 5 (38%) from the hand. The degree of antibiotic and virulence profile diversification within this PFGE cluster was very high with 9 different toxin genotypes. The most prevalent *spa* type was t026 whereas the *agr* group 3 was dominant. This pattern was characteristic of 2 among the multidrug-resistant strains and 8 among those resistant to linezolid. The strains resistant to three antibiotics were isolated from the nares and one of them (2D) also carried four toxin genes. The pattern B grouped 5 nasal strains with the same genotypic profile in terms of toxin genes (*sea-tst-sem-sec*), antibiotic resistance determinants (PEN-LZD) and *spa* type (t030). Four strains showed the macrorestriction pattern C. Among them, one was isolated from the hand and was erythromycin resistant. Two strains with the same genotypic profile were isolated from the same handler but in different years. One strain was classified as multidrug-resistant with a different toxin profile. All 6 strains associated to the PFGE type D were isolated from the nose and 5 different toxin and antibiotic resistance genotypes were found. Strains included in this pattern showed a high number of toxin genes and low antibiotic resistance. All of these data are showed in table 2.

4. Discussion

The prevalence of carriers (33%) corresponded to previously reported studies (Alhashimi Ahmed, & Mustafa, 2017; Jordà, Marucci, Guida, Pires, & Manfredi, 2012; Hatakka et al., 2000). Two handlers were again found positive to *S. aureus* after treatment with mupirocin and were therefore classified as persistent carriers. A single strain reporting the same antibiotic and toxin profile was isolated from the nose of each persistent carrier during the 2015. The occurrence of the same strain, colonising both nasal and hands among different handlers, suggested a possible *S. aureus* transmission and was an indication of a potential risk for product contamination during manufacturing and packaging.

The identification of four different macrorestriction patterns among seven handlers indicated the large variety of circulating *S. aureus*. The enterotoxigenic strains were widely distributed among the PFGE types and the high prevalence of strains carrying enterotoxin genes was in accordance with other studies (Bergdoll, Crass, Reiser, Robbins, & Davis, 1981; Hatakka et al., 2000). In this study, the toxin gene *sem* was the most frequent, which is usually not the case. van Belkum et al. (2006) have previously reported a similar occurrence. Generally, the majority of staphylococcal food contaminations are associated to SEA toxin (Wieneke, Roberts, & Gilbert, 1993; Cha et al., 2006) largely described in invasive infections. Conversely, SEM toxin,

belonging to the locus *egc* (enterotoxin gene cluster) highly present in *S. aureus* isolates, is not associated with severe infections (van Belkum et al., 2006). However, the even presence of the *sem* and *egc* toxin genes in samples from nasal carriers may be explained by the lower immunogenicity of the respective gene products that would therefore be more tolerated. The high variability in the distribution of *sem*, alone or with other toxin genes, allowed the identification of a potential relation between one virulence profile ($sea^{+}tst^{+}sem^{+}sec^{+}$) with one antibiotic resistance pattern (PEN-LZD). However, this possible association needs to be supported with more cases. Currently, little information is available for food poisoning by strains with unfrequent gene toxins and harbouring the *egc* cluster (Ikeda, Tamate, Yamaguchi, & Makino, 2005; van Belkum et al., 2006). The correlation between high number of toxin genes and multidrug resistance is frequently reported (Karimi et al., 2017; Moshtagheian et al., 2018). However, the different origins of strains undermine a valid reconstruction of the evolutionary process hence affecting our understanding of this association (Luo et al., 2018). Moreover, the frequency of each and every SE gene among *S. aureus* strains from different countries is extremely variable and the majority of them are located on mobile genetic elements (Grumann, Nübel, & Bröker, 2014). In light of this, no association between virulence traits and antibiotic resistance is conclusive.

A notable level of antimicrobial resistance rate was found in this work and many strains exhibited multiple drug resistance. No methicillin-resistant *S. aureus* (MRSA) strains were detected reducing the potential risk associated to the consumption of the products. On the other hand, the findings from the present work are in agreement with the high penicillin and erythromycin resistance rates recorded in staphylococci worldwide (Lowy, 2003; Schlegelova et al., 2008). These antibiotics are largely used in human medicine and the growing resistance rate poses big concerns to the therapy of *S. aureus* infections. In our study, erythromycin resistance was associated to *erm*C and *msr*A, which is consistent with the findings of Spiliopoulou, Petinaki, Papandreou, & Dimitracopoulos (2004) who reported these genes as predominant among erythromycin-resistant MSSA. High rate of linezolid resistance found among carrier handlers is noteworthy. Linezolid-resistant *S. aureus* was reported by many and different surveillance programmes. In Italy, linezolid resistance is higher within the coagulase-negative group of staphylococci (Gu, Kelesidis, Tsiodras, & Hindler, 2012), even if its rapid spread among *S. aureus* has been recently described (Bongiorno et al., 2010). The linezolid resistant isolates from this work exhibited low level resistance by the disk diffusion test. As a confirmation, molecular analysis showed that the mechanism of resistance was associated to the commonest point mutation mapping in the 23S rDNA sequence. Conversely, high-level resistance to linezolid is commonly due to the expression of the *cfr* gene (Long and Vester, 2012).

Among our isolates we have identified *agrI*, *agrII* and *agrIII* types. Generally, the lack of *agrIV* was associated to the MRSA strains (Azimian, Najar-pirayeh, Mirab-Samiee, & Naderi, 2012; Manago et al., 2006), but this study demonstrated that it might be well associated to MSSA. The most prevalent group was *agrIII,* followed by the *agrI,* and this is in agreement with the results of other studies (Ben Ayed, Boutiba-Ben, Boubaker, Samir, & Ben Redjeb, 2006). In facts, the occurrence of *agrIII* and *agrI* among MRSA strains has been also well documented (Azimian et al., 2012) suggesting that there is no definite correlation between the status of methicillin

susceptibility and *agr* typing. The *agr* locus was recognised as the regulator of *S. aureus* virulence factors and its association with specific infection has been reported by several studies. Infective endocarditis has been frequently associated to *agrII* and the Toxic Shock Syndrome 1 (TSST-1) to the *agrIII* (Jarraud et al., 2002). Invasive infections, such as bacteraemia, were associated to the *agrI* (Ben Ayed et al., 2006), while strains responsible for the Staphylococcal Scalded Hand Syndrome (SSSS) were often positive to *agrIV* (Jarraud et al., 2000). In this study, a correlation between *agr* groups and toxin genes' profiles was not identified.

In order to assess the clonal relationship between strains at a greater resolution, all isolates were typed by means of the *spa*-typing scheme. It is known that *spa* types distribution is geographically and temporally distinct and, given that distinct *spa* types share similar repeats, strains are considered as genetically related (Taeksoo, Jongyoun, Ki Ho, Jeong-Su, & Eui-Chong, 2011). Interestingly, 7 different known *spa* types were found, confirming the high discriminatory power of this method. The most common was t030 that is frequently detected among MRSA strains of human origin (Grundmann et al., 2010). Importantly, the fact that strains sharing other genotypic features were identified in different *spa* types suggests that evolution of the *spa* short sequence repeats was not following the evolution of the complete genome (Ruppitsch et al., 2006). This study confirmed the high discriminatory power of *spa* typing, compared to that of PFGE, to portray strains genetic fingerprints, which is associated with the high level of polymorphism within the protein A gene X region. Moreover this method is fast and data output easily comparable on a worldwide basis.

5. Conclusions

In conclusion, the high prevalence of carriers among the food handlers could be a potential source of product contamination. The occurrence of strains with the potential of producing virulence factors and showing multidrug resistance may greatly contribute to the increase in the incidence of serious foodborne diseases. The isolation of the same strain from different handlers highlights the importance of the hygiene education as an efficient preventive measures. Finally, the results showed that the application of carriage status monitoring programme among food handlers could be a very important tool to preserve the safety of the product.

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Table 1. Carriage state of the positive food handlers during the three years period.

Food handler No.	Carriage status per year						
	2013			2014		2015	
	I[a]	II[b]	III[c]	I[a]	II[b]	I[a]	II[b]
1	+	+	+	+	-	-	-
2	+	+	+	+	+	+	-
3	+	-	-	-	-	-	-
4	+	+	-	-	-	-	-
5	+	+	+	+	+	+	-
6	-	-	+	+	-	-	-
7	+	+	-	-	-	-	-

[a]I,[b]II,[c]III: first, second and third collection of the year

Table 2. Typing profiles of *S. aureus* strains isolated from anterior nares and hand of food handlers

Strains[a]	Isolation	Year	Toxin gene profile	atb resistance profile[b]	*agr* group	*spa* type	PFGE type
1A	nose	2013	*tst-sem*	PEN-ERY	3	t10307	
1AA	hand	2013	*sea-sem-sec*	FC-LZD	3	t026	
2A	hand	2013	*sem*	LZD	1	t026	
1B	nose	2013	*tst-sem-sec*	PEN-LZD-ERY	3	t10307	
2AA	nose	2013	*sea-tst-sem-sec*	PEN-LZD	3	t026	
3A	nose	2013	*sem*	LZD	1	t026	
4A	nose	2013	*sea-tst-sem-sec*	PEN-LZD	1	t030	A
3AA	hand	2013	*sem*	PEN-ERY	1	t026	
1BB	hand	2013	*sem-seo*	LZD-ERY	1	t030	
1D	nose	2013	*sem*	PEN-ERY	3	t012	
1E	hand	2014	*sem*	PEN-ERY	3	t1192	
2D	nose	2014	*tst-sem-sec-seo*	PEN-LZD-DA	3	t026	
5F	nose	2015	*sea-tst-sec-seo*	PEN	3	t026	
5A	nose	2013	*sea-tst-sem-sec*	PEN-LZD	3	t030	
4B	nose	2013	*sea-tst-sem-sec*	PEN-LZD	3	t030	
5B	nose	2013	*sea-tst-sem-sec*	PEN-LZD	3	t030	B
5C	nose	2013	*sea-tst-sem-sec*	PEN-LZD	2	t030	
5D	nose	2014	*sea-tst-sem-sec*	PEN-LZD	1	t030	
1DD	hand	2013	*sem*	ERY	1	t030	
6A	nose	2013	*sem-seo*	PEN-LZD	2	t209	C
6B	nose	2014	*sem-seo*	PEN-LZD	2	t209	
2E	nose	2014	*sea-sem*	PEN-LZD-ERY	2	t030	
2B	nose	2013	*sea-tst-sem-sec-sei*	PEN-LZD	2	t026	
7A	nose	2013	*sem*	PEN-LZD	2	t002	
7B	nose	2013	*sem*	PEN-LZD	2	t002	
2C	nose	2013	*tst-sem-sec-seo*	PEN	2	t026	D
5E	nose	2014	*sem-sec-seo*	PEN-ERY	3	t030	
2F	nose	2015	*sea-tst-sec-seo*	PEN	2	t030	

[a]Strains names consist of the number of handlers followed by letters differentiating the isolation source (example: 1A was the nasal strain isolated from the handler 1 and the strain 1AA was isolated from the hand of handler 1);

[b]PEN=penicillin; LZD=linezolid; ERY=erythromycin;FC=fusidic acid; DA=clindamycin;

Table 3. Repeat successions and frequencies detected in this study

spa type	Repeat Succession	No of isolates (n=28)
t030	r15-r12-r16-r02-r24-r24	11
t012	r15-r12-r16-r02-r16-r02-r25- r17- r24-r24	1
t10307	r15-r12-r16-r02-r16-r435-r25-r17-r24-r24	2
t002	r26-r23-r17-r34-r17-r20-r17-r12-r17-r16	2
t209	r07-r16-r12-r23-r34	2
t026	r08-r16-r34	9
t1192	r15	1

Highlights

Staphylococcus aureus is a commensal human pathogen colonizing the nose and the skin.

Asymptomatic carriers among handlers can be a potential source of food contamination.

Monitoring the carriage status of food handlers can increase the food safety.

Evaluation of the risk for the consumer through the strains molecular typing.