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Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen emerging in hospitals as well as community and livestock. MRSA is a significant and costly public health concern because it may enter the human food chain and contaminate milk and dairy products causing foodborne illness.

This study aimed to determine the occurrence and the characteristics of MRSA isolated from 3760 samples of milk and dairy products in a previous survey conducted in southern Italy during 2008–2014. Overall out of 484 *S. aureus* strains isolated, 40 (8.3%) were MRSA and were characterized by *spa*-typing, Multi-Locus Sequence Typing, SCC*mec* typing, Staphylococcal enterotoxins (SEs) genes, Panton-Valentine Leukocidin (PVL) genes and ability to form biofilm.

The most frequently recovered STs were ST152 (t355–67.5%), followed by ST398 (t899, t108–25%), ST1 (t127–5%) and ST5 (t688–2.5%). All isolates harboured the SCC*mec* type V (92.5%) or IVa (25%). In one isolate (2.5%), ST398/t899, the SCC*mec* resulted not detected. Three isolates (7.5%) carried one or more enterotoxin encoding genes (one strain had *seg*, *sei*, *sem*, *sen* and *seo* genes; two strains had *seh* gene). The 50% of isolated strains harboured PVL-encoding genes. Molecular analysis for *icaA* and *icaD* genes showed: 72.5% *icaA* and *icaD* positive, 25% only *icaD* gene and one *icaA* and *icaD* negative. The detection of MRSA in food of animal origin is a potential health hazard, thus it is necessary monitoring of food-producing animals and improving hygiene standards in food practices in order to reduce the microbiological risk to minimum.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognised as major cause of healthcare-associated infections worldwide. MRSA strains appear to have been transferred from health care settings into the community and have emerged as particularly associated with community-associated infections in humans (Scientific Report of EFSA and ECDC, 2015). Moreover, in recent years, MRSA has been identified as an emerging pathogen in livestock and companion animals, as well as some other farm animal species (Antoci et al., 2013).

Hospital-associated MRSA and community-associated MRSA are those strains predominantly affecting humans, and these generally do not involve food-producing animals; however, livestock-associated MRSA may also be harboured by humans, especially where there is occupational contact with affected livestock. Livestock-associated MRSA may cause illness in humans, although transmissibility between humans has been shown to be very limited, even in healthcare facilities (Scientific Report of EFSA and ECDC, 2015).

In recent years, MRSA strains have been recovered from several animal-source food such as poultry, pork and beef, suggesting that foods may serve as reservoir and source of community-associated MRSA (Wang et al., 2014). Animals could act as a source of *S. aureus* zoonotic infections, especially for those clones seeming to lack specific host tropism, while humans could represent an important source of new pathogenic strains affecting livestock (Luini et al., 2015).

In addition, *S. aureus* is considered one of the most important causative infective agent of mastitis in dairy cattle (Moon et al., 2007; Nam et al., 2011). Generally, methicillin is not used for treatment of mastitis but MRSA has been recovered from dairy cattle and it has been isolated from milk. Moreover the presence of MRSA in the environment might also be one of the sources of MRSA infection in animals since it may survive for several months (Lim et al., 2013).

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The aim of this study was to 1) assess the occurrence of MRSA isolated from milk samples and dairy products collected from 2008 to 2014 in southern Italy, 2) provide molecular characterization of MRSA isolates, with respect to virulence-associated genes and ability to form biofilm, 3) provide useful data about the presence of MRSA strains in food for epidemiological studies.

2. Methods

2.1. Sampling

A total of 3760 milk samples and dairy products were analyzed from 2008 to 2014 by the Food Microbiology Laboratory of the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (IZS PB) in Foggia. The samples were collected from several dairies in the Apulia region by the local health services and transferred under refrigerated conditions (at approximately 4 °C) to the laboratories of IZS PB for the detection of *S. aureus* according to national official monitoring activities and regional agreements with an average annual of 537 samples. All samples were processed on the day of collection.

2.2. Isolation and identification of S. aureus

The samples were processed according to EN ISO 6888 1-2 1999 and for each sample, five colonies with the typical aspect of coagulase-positive staphylococci were identified as *S. aureus* using CHROMagar™ (CHROMagar, Paris, France). The DNeasy Blood & Tissue Kit (Qiagen S.r.l., Italy) was used to extract bacterial DNA, following the manufacturer's instructions. The extracts were subjected to a multiplex PCR assay for the detection of 16S rRNA (Monday and Bohach, 1999), *nuc* (Costa et al., 2005) and *mecA* (Murakami et al., 1991) genes in order to confirm the identification of *S. aureus* and to test isolates for methicillin resistance. Strains positive for *mecA* gene (one strain per sample) were subjected to further molecular characterization. Reference strains of *S. aureus* were used as positive (ATCC 33591) and negative (ATCC 25923) controls.

2.3. MRSA phenotypic confirmation

To verify phenotypically the presence of *mecA* gene, an oxacillin disk diffusion susceptibility test was performed with 1 μg oxacillin disk (Liofilchem s.r.l., Teramo, Italy) on Muller-Hinton agar, following the CLSI guidelines (CLSI, 2012). Mueller-Hinton agar plates (Biolife Italiana, Milano, Italy) were inoculated with a suspension (equivalent to a 0.5 McFarland standard) of each *mecA* positive strain. Plates were incubated at 37 °C and the results were recorded after 24 h. Following breakpoints were considered: oxacillin resistant ≤10 mm, intermediate 11–12 mm, susceptible ≥13 mm (CLSI, 2012). At the same time, the strains positive for *mecA* gene were plated on CHROMagar™ MRSA (CHROMagar, Paris, France). After 18–24 h of incubation at 37 °C, the colonies which appeared pink to mauve were considered MRSA.

2.4. SCCmec-, spa- and multi locus sequence typing

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was carried out discriminating the *mec* complex and the *ccr* genes complex as described elsewhere (Kondo et al., 2007). Subtyping of the SCC*mec* type IV was obtained applying the method described by Zhang (Zhang et al., 2012).

Amplification of the polymorphic region of the protein A gene (*spa* typing) was performed according to the protocol of Strommenger (Strommenger et al., 2006). Multilocus sequence typing (MLST) was obtained as described elsewhere (Enright et al., 2000). Alleles and ST were assigned by submitting the DNA sequences to the *Staphylococcus* MLST database (http://saureus.mlst.net/).

2.5. Detection of virulence-associated genes and ability to form biofilm

The detection of 12 genes encoding staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sem*, *sen* and *seo*), was performed using twelve specific primer sets (Jarraud et al., 2002; Løvseth et al., 2004; Boerema et al., 2006; Monday and Bohach, 1999; Rosec and Gigaud, 2002) combined in three multiplex-PCR assays. The *sec* primers were able to detect all three subclasses of enterotoxin SEC (C1, C2 and C3) (Løvseth et al., 2004). The ability to form biofilm was tested according to Zmantar et al. (2008). In addition, biofilm production by MRSA strains was determined using a semi-quantitative adherence assay by microtiter plate (MTP) as described previously (Zmantar et al., 2010). The protocol provided the measurement of optical density (OD) at 570 nm of adherent biofilm stained with 1% (w/vol) crystal violet. Biofilm formation was categorized as highly positive ($OD_{570} \geq 1$), low grade positive ($0.1 \leq OD_{570} < 1$), or negative ($OD_{570} < 0.1$). The presence of *lukF-PV* and *lukS-PV* encoding Panton-Valentine Leukocidin (PVL) was evaluated using the primers described by Hesje et al. (2011) (Fig. 1). Reference strains of *S. aureus* ATCC 13565 (*sea*, *sej*), ATCC 14458 (*seb*), ATCC 19095 (*sec*, *seh*), ATCC 23235 (*sed*, *seg*, *sei*, *sem*, *sen*, *seo*), ATCC 27664 (*see*), ATCC 25923 (*icaA*, *icaD*, *lukF-PV* and *lukS-PV*) were used as positive controls. *S. aureus* ATCC 6538 (no enterotoxigenic strain) and *S. epidermidis* ATCC 12228 were used as negative controls.

3. Results

Overall 3760 milk samples and dairy products, collected from 2008 to 2014, were subjected to bacteriological analysis. Out of 484 (484/3.760; 12.9%) *S. aureus* strains isolated, 40 (40/484; 8.3%) were MRSA. PCR assay for *mecA*, disk diffusion test with oxacillin and CHROMagar™ MRSA (CHROMagar, Paris, France) confirmed methicillin resistance of isolates.

Five different *spa*-types and 4 different STs were identified from characterization of the MRSA isolates. The most frequently recovered ST (27/40; 67.5%) was ST152 (t355), followed by ST398 (t899, t108) (10/40; 25%), ST1 (t127) (2/40; 5%) and ST5 (t688) (1/40; 2.5%).

The most frequently recovered *spa*-type was t355 (27/40; 67.5%), followed by t899 (8/40; 20%), t108 and t127 (2/40; 5%) and t688 (1/40; 2.5%). All isolates harboured the SCC*mec* type V (37/40; 92.5%) or IVa (2/40; 5%). In one isolate (1/40; 2.5%), ST398/t899, the SCC*mec* resulted not detected.

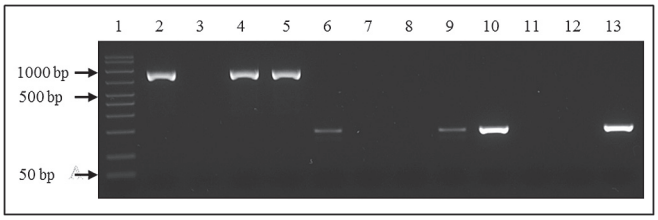

Fig. 1. Strains containing Panton-Valentine Leukocidin and *icaA*/*icaD* genes. Lane 1: molecular size standard; lane 2: ATCC 25923 (PVL positive); lane 3: negative control; lane 4: sample #15; lane 5: sample #22; lane 6: ATCC 25923 (*icaA* positive); lane 7: negative control; lane 8: sample #15; lane 9: sample #22; lane 10: ATCC 25923 (*icaD* positive); lane 11: negative control; lane 12: sample #15; lane 13: sample #22.

Three isolates of 40 (7.5%) carried one or more enterotoxin encoding genes (one strain had *seg*, *sei*, *sem*, *sen* and *seo* genes; two strains had *seh* gene).

The evaluation of the presence of PVL-encoding gene showed that 50% (20/40) of isolated strains had *lukF-PV* and *lukS-PV* genes.

The *icaA* and *icaD* genes were detected both in 29 isolates (29/40; 72.5%), while in 10 isolates only *icaD* gene was detected (10/40; 25%). Only one isolate was *icaA* and *icaD* negative. Among these 40 *S. aureus* strains, 15 (15/40; 37.5%) were slime producers developing almost black or very black colonies on CRA (Congo red agar) plates and the remaining 25 strains were non-producers developing red or bordeaux colonies. As regard the biofilm formation of MRSA strains, 97.5% (39/40) of the isolates were biofilm producers with MTP method, although production level varied. Among these, 15% of isolates (6/40) were strongly biofilm producer, 82.5% (33/40) were low grade biofilm positive and only one was biofilm negative. Comparison of results obtained by CRA method versus that of MTP method showed that out of 25 non-producers on CRA, 24 isolates were biofilm producers but with different degrees of production (23 low grade producers and one strongly producer) and only one isolate was true negative by both methods.

All the listed characteristics are showed in Table 1.

4. Discussion

This study reports on the occurrence of MRSA collected from milk samples and dairy products and the molecular characteristics of MRSA isolates, with respect to virulence-associated genes and ability to form biofilm.

MRSA is a significant public health concern given its ability to contaminate food of animal origin and to colonize and infect humans and animals (Petinaki and Spiliopoulou, 2012). Both HA-MRSA and LA-MRSA are a common cause of bovine mastitis and thus contaminate milk and dairy products which can be considered vehicles of transmission of MRSA to humans (Mancini et al., 2015).

In this survey, 12.9% of samples were contaminated by *S. aureus* and the 8.3% were MRSA. Many studies have evaluated the prevalence of MRSA in milk, but few have investigated the presence of MRSA in dairy products. Riva et al. (2015) found that the prevalence of *S. aureus* was 9.1% in raw milk and the 20% were MRSA. One study from Germany on MRSA in bulk tank milk reported that the 4.4% of the analyzed samples were positive for MRSA (Kreausukon et al., 2012). Normanno et al. (2007a) reported a contamination rate of milk and dairy products of 17% and overall 160 *S. aureus* strains isolated from food of animal origin, six (3.75%) were MRSA, derived all from milk and dairy products (Normanno et al., 2007b). The

Table 1
Genotypic characteristics, virulence-associated genes and slime factor of MRSA isolates studied.

ID #	Samples	SCC*mec*	*Spa*-type	MLST	SEs genes	*lukF-PV*/*lukS-PV*	*icaA*	*icaD*	CRA	OD_{570}[a]
1	*mozzarella* cheese	V	t108	ST398	–	–	+	+	P	++
2	*mozzarella* cheese	V	t108	ST398	–	–	+	+	P	++
3	cheese	V	t899	ST398	–	–	+	+	NP	++
4	cheese	V	t899	ST398	–	–	+	+	NP	++
5	cheese	V	t899	ST398	–	–	+	+	NP	++
6	cheese	V	t899	ST398	–	–	+	+	NP	++
7	*stracciatella* cheese	V	t355	ST152	–	+	+	+	NP	++
8	cheese	V	t899	ST398	–	–	+	+	NP	++
9	cheese	V	t899	ST398	–	–	–	+	NP	++
10	cheese	V	t355	ST152	–	+	–	+	NP	++
11	cheese	V	t355	ST152	–	+	–	+	P	++
12	cheese	V	t355	ST152	–	+	–	+	NP	++
13	cheese	V	t355	ST152	–	+	–	+	P	++
14	cheese	V	t355	ST152	–	+	–	+	P	++++
15	cheese	V	t355	ST152	–	+	–	–	NP	–
16	cheese	V	t355	ST152	–	+	–	+	NP	++
17	cheese	V	t355	ST152	–	+	–	+	NP	++
18	cheese	V	t355	ST152	–	+	–	+	NP	++
19	cheese	V	t355	ST152	–	+	–	+	P	++
20	*scamorza* cheese	V	t688	ST5	*seg, sei, sem, sen, seo*	–	+	+	NP	++
21	cheese	V	t899	ST 398	–	–	+	+	NP	++
22	cheese	V	t355	ST152	–	+	+	+	P	++
23	cheese	V	t355	ST152	–	+	+	+	P	++++
24	cheese	V	t355	ST152	–	+	+	+	P	++++
25	milk	V	t355	ST152	–	–	+	+	NP	++
26	milk	V	t355	ST152	–	–	+	+	NP	++
27	milk	V	t355	ST152	–	+	+	+	P	++
28	milk	V	t355	ST152	–	+	+	+	NP	++
29	milk	V	t355	ST152	–	+	+	+	NP	++
30	milk	V	t355	ST152	–	–	+	+	NP	++
31	milk	V	t355	ST152	–	+	+	+	NP	++++
32	milk	V	t355	ST152	–	+	+	+	P	++
33	milk	V	t355	ST152	–	–	+	+	NP	++
34	milk	V	t355	ST152	–	–	+	+	P	++++
35	milk	V	t355	ST152	–	–	+	+	P	++++
36	milk	V	t355	ST152	–	+	+	+	NP	++
37	milk	V	t355	ST152	–	–	+	+	NP	++
38	milk	ND	t899	ST 398	–	–	+	+	NP	++
39	milk	IVa	t127	ST1	*seh*	–	+	+	P	++
40	milk	IVa	t127	ST1	*seh*	–	+	+	P	++

CRA, Congo red agar; ND, not detected; NP, non producer; P, producer; SEs, Staphylococcal enterotoxins.
[a] Strongly biofilm positive (++++), low grade biofilm positive (++) and biofilm negative (–).

recovery of MRSA strains (8.3%) among our isolates should be considered as a potential public health risk since this pathogen may enter the food chain. For this reason it is necessary to monitor the health status of animals and improve the hygienic conditions of milking, washing and systematic disinfection of plants as well as staff education involved in the production chain.

In this study, the most common MRSA isolates belonged to a clone characterized by t355/ST152/SCC*mec* type V (67.5%). In addition, the 74.1% of these clones was PVL-positive. PVL-positive MRSA with t355/ST152 genotype corresponds to a CA-MRSA clone known to be responsible for human infections, and reported in several countries, including Italy (Monaco et al., 2014). It was first described in Slovenia in 2004 as a CA-MRSA and isolated from a football player affected by Skin and Soft-Tissue Infections (SSTI) (Müller-Premru et al., 2005). Monecke et al. (2007) found the same genotype in an immigrant child from Macedonia. In Italy, a t355/ST152 MRSA clone isolated from a patient coming from Albania was responsible for a small outbreak in an intensive care unit of a children's hospital (Ugolotti et al., 2011).

This report is one of the few describing PVL-positive MRSA belonging to a CA-MRSA clonal lineage in milk and dairy products with the t355/ST152 genotype. In North Italy, a PVL-positive MRSA with the same genotype was recovered in bovine milk (Benedetti et al., 2010).

PVL-positive CC152-MRSA-V has been found sporadically in Germany, Sweden, Switzerland and Australia and it was observed that some patients infected with this strain had ties to Balkan countries (Macedonia, Kosovo). Probably, this clonal lineage has emerged in Balkans where there is a wider distribution with respect to other regions (Monecke et al., 2011).

In Apulia (South Italy), it may have been introduced by colonizer/infected farm workers coming from the Balkans although one cannot exclude that this clone can be a colonizer of cows in certain areas (Mancini et al., 2015).

The second most recovered MRSA belonged to ST 398 (25%), *spa*-type t899-t108, SCC*mec* type V, PVL-negative which is considered an important livestock-associated lineage mainly related to pig farming (Antoci et al., 2013). PVL-negative ST398-MRSA-V has been observed also in humans, cattle, horses, dogs, poultry, chickens and turkeys and found in retail meat of different domestic animals (Monecke et al., 2011). Benedetti et al. (2010) recovered, in bovine milk, 9/14 PVL-negative ST398-MRSA-V. In a survey conducted in the Netherlands, t899/ST398/SCC*mec*V was found in milk samples, while it has been described in Belgian cows causing clinical and subclinical mastitis (Tavakol et al., 2012; Vanderhaeghen et al., 2010). These evidences suggest that MRSA ST398 could be transmitted among various animal species and cause bovine intramammary infections (Tavakol et al., 2012). Little is known about the presence of ST398-MRSA-V in dairy products but it was detected in a previous study conducted in Italy (Crisetti et al., 2012).

Other STs recovered were ST1 (5%) *spa*-type t127, SCC*mec* type IVa, *seh* positive, PVL-negative and ST5 (2.5%) *spa*-type t688, SCC*mec* type V, *seg*, *sei*, *sem*, *sen*, *seo* positive, PVL-negative.

ST1 is considered a frequent clone in human infections which can cause bovine mastitis and is associated with pig carriage in Italy and other European countries. According to phylogenetic studies, some animal *S. aureus* lineages derived from human lineages. This resulted in the loss of some virulence factors that were useless in the new host and the acquisition of new characteristics (Pantosti, 2012). In Hungary, MRSA were recovered in 4% of samples from cows with subclinical mastitis. This clones belonged to ST1, *spa*-type t127, of likely human origin, and the transmission of which to a farm worker in close contact with bovines was also demonstrated (Juhász-Kaszanyitzky et al., 2007). In a long-term study in a dairy cow with an intramammary infection, t127/ST1/MRSA was recovered from milk samples. This finding suggested a human-cow transmission of this MRSA type since the cow was from a closed herd and had never been moved (Pilla et al., 2012). In a survey about MRSA ST1, the bovine isolates shared 90%–100% similarity with human isolates and were t127/ST1/MRSA/SCC*mec*IVa *seh* positive. It was observed that *seh* gene appeared to be constitutive of ST1, independently from the host origin, as previously reported (Alba et al., 2015; Monecke et al., 2011). All these findings may suggest a human-bovine exchange and the adaptation of the human MRSA to the bovine host.

MRSA with genotype t688/ST5/SCC*mec*V belongs to CC5 that is a common and widespread clonal complex, which comprises both HA- and CA-MRSA. As CC5 isolates, this clone harbours the enterotoxin gene cluster *egc* (*seg*, *sei*, *sem*, *sen*, *seo*) and is PVL-negative (Monecke et al., 2011). ST5 lineage can be considered an animal-adapted clone since it was reported in humans as well as companion animals, poultry, pigs and cattle (Pantosti, 2012).

Regarding the risk of Staphylococcal food poisoning (SFP), many different foods are implicated such as milk and cheeses, cream-filled pastries, ham, sausages, canned meat, salads, cooked meals and sandwich filling. SFP is a mild form of intoxication occurring after the ingestion of food containing from 20 ng to 1 μg of staphylococcal enterotoxins (SEs), which is enough to determine symptoms in humans (Bergdoll, 1989). Contamination can occurs after heat treatment of the food and the main sources of contamination are colonizer/infected food handlers but in some food such as raw meat, sausages, raw milk and raw milk cheese, contaminations are due to animal carriage or to infections of animal origin (Ortega et al., 2010). Today, 23 SEs have been reported in literature and only the most important enterotoxins serological types (SEA, SEB, SEC, SED, and SEE) can be identified with commercially available immunoassay kits (Nia et al., 2016). These five SEs have been shown to exhibit emetic activity (Johler et al., 2015). The 7.5% of the MRSA isolated in this study carried one or more enterotoxin encoding genes, one isolate had the *egc* cluster genes and two isolates harboured *seh* gene. The occurrence of *egc* in *S. aureus* isolated from food is quite variable depending on the geographical and/or food type origin of isolates, but it is remarkably high among clinical isolates where it is supposed to contribute to the infection process (Viçosa et al., 2013). Besides, there are evidences that SEH has been responsible for milk-based food-poisoning outbreaks (Jørgensen et al., 2005; Ostyn et al., 2012). This means that the risk of SFP can be associated to the consumption of milk and dairy products especially in the absence of strict hygiene standards along the food chain to avoid SEs. Consequently an active microbiological surveillance is needed in order to reduce the contamination rate and to ensure food safety. In literature, only few data are available on the occurrence of MRSA in SFP. Kerouanton et al. (2007) reveled two MRSA strains of the 33 isolated strains of *S. aureus* implicated in SFP outbreaks. Another evidence that MRSA may also be involved in SFP outbreaks was reported by Jones et al. (2002). Despite the few reported cases, it can not be excluded the risk for humans of infections caused by enterotoxigenic MRSA. Moreover, the detection of MRSA in foods of animal origin may constitute a risk for consumers and especially for immunocompromised individuals. In immunocompromised persons the specific and non-specific immune responses are not able to act as barriers to prevent colonization of the gastrointestinal tract and ingestion of food contaminated by MRSA may lead to sometimes lethal disease (Normanno et al., 2007b).

Panton-Valentine Leukocidin (PVL) is a toxin causing the lysis of leukocytes associated with severe skin and soft tissue infection and highly necrotizing pneumonia. This toxin is typically associated with CA-MRSA clones suggesting that methicillin resistance has contributed to the success of PVL-positive *S. aureus* strains

(Boyle-Vavra and Daum, 2007). The presence of *lukF-PV* and *lukS-PV* genes in the 50% of isolates of this survey, is quite unusual because they are characteristic of a clone typically human as it is uncommon to find a clone ST152 in milk and dairy products. PVL was previously described in CA-MRSA isolated from milk samples in Minnesota and Korea (Haran et al., 2012; Kwon et al., 2005). This finding could be explained considering that some animal *S. aureus* lineages originated from human strains following genetic modifications and acquired new pathogenicity factors (Pantosti, 2012).

The ability of some strains to form biofilm is considered to be a significant virulence factor because established biofilms can tolerate antimicrobial agents, thus making the bacterium extremely difficult to eradicate (Basanisi et al., 2015). Biofilm formation is regulated by expression of polysaccharide intracellular adhesion (PIA), which mediates cell to cell adhesion and is the product of *ica* locus containing *icaA*, *icaB*, *icaC*, *icaD*. *icaA* gene encodes *N*-acetylglucosaminyltransferase and *icaD* has an important role in expression of this enzyme (Ciftci et al., 2009). Furthermore, PIA has been identified as the main exopolysaccharide component of the staphylococcal biofilm matrix (Arciola et al., 2015). In this study, the *icaA* and *icaD* genes were detected both in 72.5% of the isolates, while in 25% of the isolates only *icaD* gene was detected. Among the MRSA strains, 37.5% were slime producers on CRA plates *in vitro*, while 97.5% were biofilm producers by MTP method. Difference between results of CRA and MTP methods can be attributed to the fact that phenotypic expression of biofilm formation is highly sensitive to *in vitro* condition and hence can be detected variably by different methods (Darwish and Asfour, 2013). A good concordance was found between the PCR results and the MTP method, otherwise between PCR and CRA method. Overall, these results indicate that molecular identification of *ica*-genes and phenotypic tests should be appropriately associated for an accurate identification of biofilm producing strains.

Recently, Mirani et al. (2013) found that 98.3% of foodborne MRSA isolates carried the *icaA*/SCC*mec* IV profile. The author noticed the relation of SCCmec type IV and biofilm formation. Other authors reported also an association of SCC*mec* type V and *icaA* gene (Parisi et al., 2016; Kim et al., 2015).

Slime factor represents a potential problem for dairy industry since bacteria, embedded in their own matrix, can be resistant to the usual sanitation processes. This requires careful consideration of the sanitation methods used on farms and in the food industry.

The data obtained from this survey show that only small amounts of MRSA contaminate milk and dairy products from southern Italy and molecular typing is a very useful tool in investigating the characteristics of MRSA strains, but further studies should be conducted to monitoring the presence and evolution of these pathogens.

The role of food as a vehicle of human MRSA colonization or infection is deemed to be low by the European Food Safety Authority (EFSA, 2009). However food-related outbreaks of MRSA infection and intoxication have been formally demonstrated in several occasions (Jones et al., 2002; Kluytmans et al., 1995) and contaminated food commodities can contribute to the worldwide dissemination of clones of CA-MRSA (Herrera et al., 2016). Furthermore, there is likewise powerful evidence that human consumption of food carrying antibiotic-resistant bacteria has resulted, either directly or indirectly, in acquisition of antibiotic-resistant infections (Marshall and Levy, 2011).

The detection of MRSA in food of animal origin is a potential health hazard related to some factors such as the number of colonizer/infected food handlers, the level of hygiene in food plants and transport systems, and the level of raw milk contamination; thus it is necessary monitoring of food-producing animals and improving hygiene in food practices in order to limit the spread of the microorganism and reduce the microbiological risk to minimum.

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