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MRSA in swine, farmers and abattoir workers in Southern Italy

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important medical issue, since it causes serious and sometimes fatal infections in humans. Intensively reared swine may serve as reservoirs for MRSA that can infect swine workers, and also consumers (via contaminated meat). In this study, MRSA strains were isolated from 55 of the 85 (64.7%) intensive pig farms surveyed, and prevalence was greater on pig fattening farms than on breeding farms. In addition, we included in the study 63 foreign pigs imported for slaughter. Overall, the prevalence of MRSA in the 418 sampled swine was 59.1%; 12 genotypes were identified among the isolates; ST398 (96.4%) was most prevalent, followed by ST97 (2%), ST9 (0.8%) and ST1 (0.8%). MRSA isolates were also detected in 26 (17.3%) of the 150 operators included in the study; the genotypes detected were ST398 (85%), ST9 (7.6%), ST5 (3.8%) and ST1 (3.8%). All the strains were *pvl* negative and *pia* positive. Both swine and human strains displayed a multi-resistance pattern, and almost all were resistant to tetracycline. The results obtained in this study confirm the high prevalence of MRSA in swine reared and slaughtered in Italy, and underline the public health risk linked to the spread of antimicrobial-resistant *Staphylococcus aureus* among intensively reared pigs.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen responsible for hospital (HA-MRSA) and community-acquired (CA-MRSA) infections worldwide (Kluytmans-Vandenbergh and Kluytmans, 2006; Livermore, 2000).

Over the last decade, a clone CC398 (ST398) strictly associated with swine and farm animals (Livestock-Associated, LA-MRSA) has emerged. Although this strain does not affect livestock production, it can colonize and cause severe infections in humans (Voss et al., 2005; van Loo et al., 2007; EFSA, 2009a; Cuny et al., 2010). Since its first isolation, MRSA ST398 has attracted global attention, and reports have documented its presence in many countries in Europe, North America, and Asia (Cui et al., 2009; Khanna et al., 2008; Lim et al., 2010; Parisi et al., 2017). Furthermore, recent studies indicate that other Clonal Complexes (e.g.: CC5, CC9, CC97) classified as LA-MRSA can be pathogenic to humans and must therefore be closely monitored (Köck et al., 2010). LA-MRSA clones are circulating among human populations. They have been isolated from field staff (e.g. farmers and veterinarians) and from people without recent exposure to livestock farms, suggesting the existence of other transmission routes in addition to direct contact (Petinaki and Spiliopoulou, 2012) such as the environmental and the human to human transmission. In fact, Larsen reported an increasing number of diseases due to MRSA CC398 in/patients without direct contact with livestock (Larsen et al., 2015). An important role is played by the airborne transmission of LA MRSA in animal production environment, because MRSA could be present in the dust and inhaled by the operators working in the food animal production chain (EFSA, 2007). MRSA has been isolated from different foods, such as milk, beef, chicken and pork (Normanno et al., 2007, 2015; Kitai et al., 2005; Known et al., 2006; de Boer et al., 2009; O'Brien et al., 2012; Parisi et al., 2016). Thus, the handling and consumption of contaminated food is considered a potential source of colonization/infection for humans (EFSA, 2009a; Larsen et al., 2016). In Italy, the fifth largest European producer of pig meat (EUROSTAT, 2017), approximately 14 million pigs are slaughtered for meat each year and over 4,000 people work in the pork production chain (breeding, slaughtering and cutting) (ISTAT, 2016). Normanno et al., reported that 37.6% of pigs coming from different countries imported to Italy for slaughter were MRSA carriers and some of the isolated clones were shared with the abattoirs workers

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(Normanno et al., 2015). However, the spread of MRSA in intensively reared pigs and people working in Italy's pig industry remains unclear; the only available data refers to Northern and Central regions (EFSA, 2009b; EFSA, 2010; Battisti et al., 2010), while no data are available for the Southern regions. The aims of this report are as follows: 1) to investigate the prevalence, the genetic characteristics and the antimicrobial resistance pattern of MRSA isolated from swine bred and/or slaughtered in the studied area; 2) to evaluate the rate of colonization and the genetic characteristics of the isolates in farmers and abattoir workers who had contact with the studied swine.

2. Materials and methods

2.1. Sampling design

From December 2014 to November 2015, a survey was carried out in 85 pig farms situated in Southern Italy, across Apulia and Basilicata regions: 10 pig fattening farms and 75 breeding farms. The number of pigs tested on each farm was calculated on the basis of the total number of animals under a specified expected prevalence (25%) and desired confidence (95%) (Thrusfield, 1995) up to 8 pigs in the farms with a number of animals ranging between 1 and 30 and 10 pigs in larger farms with a number of pigs included between 31 and 10.000. A stratified random sampling was performed in farms in which different buildings or animal categories were reared.

Nasal swabs were collected from 355 pigs (1 swab per animal) on 85 farms and from 130 farm workers (1 swab per worker) on 79 farms. In addition, 63 nasal swabs were analyzed from imported pigs. Sampling was carried out on 10% of the pigs in each foreign batch on the sampling day on arrival at the abattoir. In addition, 20 swab samples were collected from abattoir workers (1 swab per worker). Sterile single swabs in tubes with Amies Charcoal Medium (Amies agar transport swabs, Copan, Brescia, Italy) were used for the sampling procedure. Each swab was collected from both nostrils of the selected pigs or abattoir workers, and sampling was performed by gently rotating the swab approximately 10 times in each nostril. Swabs were then stored at 4 °C and transported directly to the laboratory for analysis within 12 h of collection.

2.2. Detection and identification of MRSA

Each nasal swab was inoculated to Mueller-Hinton broth (BiolifeItaliana, Milan, Italy) supplemented with 6.5% (w/v) NaCl (Sigma Aldrich, St Louis MO, USA). After incubation for 24 h at 35 °C, each culture was spread onto a MRSA-SELECT® plate (Bio-Rad, Marnes la Coquette, France) and incubated at 35 °C for 16–42 h (Nahimana et al., 2006). Suspected colonies were identified by conventional methods as *S. aureus*. For each positive sample, one strain was selected for further phenotypic and genotypic characterization as described below.

Oxacillin and cefoxitin disc diffusion susceptibility tests were performed with 1 μg oxacillin and 30 μg cefoxitin discs (Liofilchem s.r.l, Roseto d. A., Italy), following CLSI recommendations (CLSI M100-S22, 2012-oxacillin; CLSI M100 S24, 2014-cefoxitin). The MRSA suspension was inoculated on Oxacillin Salt Screen Agar® (Mueller-Hinton agar containing 4% NaCl and 6 μg oxacillin/ml-Biolife). Plates were incubated at 35 °C for 24 h, and any colony that grew on the plate was considered methicillin resistant (Shariati et al., 2010).

Genomic DNA was purified from the presumptive MRSA isolates using GenomicPrep® cell and a tissue isolation kit (Amersham, Piscataway, NJ, USA), following the manufacturer's instructions.

Two separate PCR assays were performed to assess the species identification and to detect the *mecA* gene, using previously described primers sau1 and sau2, and mecA147-F and mecA147-R (Zhang et al., 2005), respectively. One isolate per sample, identified as MRSA, was further characterized as described below.

2.3. Molecular typing of MRSA

The isolates were further characterized by Multi Locus Sequence Typing (MLST), *spa*-typing, Staphylococcal Chromosomal Cassette (SCC*mec*) characterization as specified below.

The PCR amplifications at the seven loci included in the MLST scheme, *arcC*, *aro*E, *glp*F, *gmk*, *pta*, *tpi* and *yqi*L, were performed as described elsewhere (Enright et al., 2000). The PCR products were purified using ExoSAP-IT according to supplier recommendations (Life Technologies). Sequence reactions were carried out using BigDye 3.1 Ready reaction mix (Life Technologies) according to the manufacturer's instructions. The sequenced products were separated with a 3130 Genetic Analyzer (Life Technologies). Sequences were imported and assembled with Bionumerics 7.6 software (Applied Maths, Belgium). Alleles and Sequence Types (STs) were assigned using Bionumerics software according to the *S. aureus* MLST database (https://pubmlst.org/saureus/).

The x region of the *spa* gene was amplified by PCR using primers *spa*-1113f and *spa*-1514r (Strommenger et al., 2006). DNA sequences were determined as described above; *spa*-types were assigned using BioNumerics 7.6 software according to Ridom spa Database (http://spaserver.ridom.de/).

Staphylococcal cassette chromosome *mec* element (SCC*mec*) typing was carried out as described by Zhang (Zhang et al., 2005).

The isolates were tested by PCR for *luk*S-*luk*F-PV, encoding Panton-Valentine leukocidin (PVL) (Hesje et al., 2011), for the *ica*A gene (intercellular adhesion) (Zmantar et al., 2008) and for *sea* to *sej* and *sem* to *seo* encoding staphylococcal enterotoxins (SEs) (Boerema et al., 2006) as described elsewhere.

2.4. Antimicrobial susceptibility testing of MRSA

Eighty-three isolates from swine, selected on the basis of their genotype and origin (every single genotype identified for each farm and every single genotype identified for foreign pigs), and all the isolates of human origin were tested for susceptibility to a panel of 20 antimicrobial agents using the disc diffusion method on Mueller-Hinton agar (Biolife), following the CLSI guidelines (CLSI VET01 S2, 2013-enrofloxacin; CLSI M100 S24, 2014-all other antibiotics). The antibiotic discs from Liofilchem were as follows: amikacin (30 μg), amoxicillin/clavulanic acid (30 μg), ampicillin (10 μg), cephalotin (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), clindamycin (2 μg), chloramphenicol (30 μg), doxycycline (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamicin (30 μg), oxacillin (1 μg), penicillin (10 μg), streptomycin (10 μg), sulfisoxazole (250 μg), tetracycline (30 μg), trimethoprim/sulfamethoxazole (25 μg), vancomycin (30 μg).

All the tests performed were carried out using the ATCC 43300 and ATCC 25923 (Biogenetics) isolates as methicillin-resistant and methicillin-susceptible *S. aureus*, respectively.

2.5. Statistical analysis

The Chi-square test (2, $P < 0.05$) with Epi Info 3.3.2 software was used to compare differences among the prevalence of MRSA in: 1) local swine vs imported swine; 2) farmers vs abattoir operators; 3) positive farms based on the type of production (pig fattening farms, breeding farms). Odds ratio with 95% confidence interval has been calculated to assess the exposure risk of farmers in MRSA positive and negative farms.

3. Results

3.1. Prevalence of MRSA in pig farms

The percentage of MRSA-positive farms was 64.7% (Table 1). MRSA colonization frequency (the proportion of farms where MRSA was

Table 1
Distribution of MRSA in pigs and in farm operators, with type of farm, and at the abattoir.

	N. of farms positive/total (%)		N. of pigs positive/total (%)	N. of operators positive/total (%)
	Pigs	Humans		
Fattening farms	10/10 (100.0)	8/10 (80.0)	71/82 (86.6)	14/19 (73.7)
Breeding farms	45/75 (60.0)	9/69 (13.0)	119/273 (43.6)	11/111 (9.9)
Abattoir			57/63 (90.5)	1/20 (5.0)
TOTAL	55/85 (64.7)	17/79 (21.5)	247/418 (59.1)	26/150 (17.3)

isolated from at least one animal) was significantly higher in fattening farms (10/10; 100%) compared to breading farms (45/75; 60%) ($p < 0.001$) (Table 1).

Farmers were found positive for MRSA in 17 of the 79 (21.5%) farms included in the study (Table 1). In eighty per cent (8/10) of fattening farms and in 13% (9/69) of breading farms at least one worker tested positive for MRSA (Tables 1 and 2). The percentage of pig farmers who tested positive for MRSA was significantly higher in pig fattening farms than breeding farms ($p < 0.001$). On the whole, operators in MRSA-positive farms had a significantly higher risk to acquire the infection (OR 12.52, 95% CI 1.6–96.3, $p < 0.01$) (Table 2).

3.2. Prevalence of MRSA in pigs

In total, 247 out of 418 (59.1%) pigs tested positive for MRSA (Table 1): 190 (53.5%) of the 355 pigs bred in Italy and 57 (90.5%) of the 63 imported pigs, tested at the abattoir. MRSA prevalence was significantly greater ($p < 0.001$) in imported pigs than in pigs bred in Italy.

3.3. Prevalence of MRSA in farmers and abattoir workers

Of 150 operators tested, 26 (17.3%) tested positive for MRSA (Table 1): 19.2% (25/130) of the farmers and 5% (1/20) of the abattoir workers. MRSA prevalence was significantly greater ($p < 0.01$) in farmers than in abattoir workers.

3.4. Genotyping of MRSA isolates

3.4.1. MRSA isolated from pigs

Genotyping analysis of 247 MRSA isolated from pigs identified the following genotypes, with a higher frequency of ST398 (t011/V, t034/V, t588/V, t899/V, t1451/V, t1456/V, t1793/V) (n = 238; 96.4%), followed by ST97 (t1730/V, t4795/V, t9301/V) (n = 5; 2.0%), ST9/t4474/V (n = 2; 0.8%) and ST1/t127/IVa (n = 2; 0.8%) (Table 3). More than one genotype was detected in each type of farm (fattening or breeding). Three genotypes, all belonging to ST398, were detected among the 57 isolates from imported pigs: ST398/t011/V (n = 28; 49.1%), ST398/t1451/V (n = 26; 45.6%) and ST398/t034/V (n = 3; 5.3%).

Table 2
Distribution of MRSA *status* of operators in MRSA positive and negative farms.

Farms		Operators		% positive
		MRSA positive	MRSA negative	
MRSA positive	Fattening	14	5	73.7
	Breeding	10	64	13.5
	TOTAL	24	69	25.8
MRSA negative	Breeding	1	36	2.7
	TOTAL	1	36	2.7

Table 3
Distribution of MRSA genotypes detected from swine and operators.

Genotype	Number of swine isolates (%)	Number of human isolates (%)
ST398/t034/V	85 (34.4%)	10 (38.5%)
ST398/t011/V	77 (31.2%)	6 (23.1%)
ST398/t899/V	40 (16.2%)	6 (23.1%)
ST398/t1451/V	32 (13%)	
ST398/t1456/V	2 (0.8%)	
ST398/t1793/V	1 (0.4%)	
ST398/t588/V	1 (0.4%)	
ST97/t1730/V	1 (0.4%)	1 (3.8%)
ST97/t9301/V	1 (0.4%)	
ST97/t4795/V	3 (1.2%)	
ST9/t4474/V	2 (0.8%)	
ST9/t1939/V		1 (3.8%)
ST1/t127/V/IVa	2 (0.8%)	1 (3.8%)
ST5/t002/N.D.		1 (3.8%)
TOTAL	247	26

3.4.2. MRSA isolated from farmers and abattoir workers

Seven genotypes were identified among the 26 MRSA isolated from farmers and abattoir workers. Of these, 85% were ST398 (n = 22) (t034/V, t011/V, t899/V), a single strain was isolated for each of the following genotypes: ST9/t1939/V, ST97/t1730/V, ST5/t002/n.d. and ST1/t127/V (Table 3). More than one genotype was detected in farmers working on fattening farms.

Genotyping analysis of the only MRSA isolate from abattoir workers identified the genotype as ST398/t034/V.

The genotypes identified from 22 (84.6%) of the 26 MRSA isolates from farmers and abattoir workers were indistinguishable from those isolated from swine sampled on the same farms and/or abattoirs.

3.4.3. Virulence factors in MRSA isolates from pigs and humans

All the tested MRSA isolates came out as PCR-negative for *luk*S-*luk*F-PV, encoding Panton-Valentine leukocidin (PVL) and PCR-positive for the *ica*A gene. Five isolates resulted PCR-positive for the presence of genes encoding staphylococcal enterotoxins (SEs): ST398/t011/V (*see*, *seo*), ST398/t034/V (*sec*) and ST1/t127/IVa (*seh*) isolated from pigs, and ST1/t127/V (*seh*) and ST5/t002/n.d. (*sed*, *sem, sej*) human isolates.

3.4.4. Antimicrobial resistance pattern of MRSA isolates from pigs and humans

All the MRSA isolates in the study were confirmed resistant using the oxacillin and cefoxitin disc diffusion test. All isolates from swine and humans displayed a multi-resistant profile. The results from the antimicrobial susceptibility tests performed on MRSA isolates from pigs and humans are reported in Tables 4 and 5. Not all the LA-MRSA strains isolated were resistant to tetracycline.

4. Discussion

Methicillin-resistant *S. aureus* (MRSA) is widespread in pigs, and ST398 (CC398) is the most frequently reported genotype on European pig farms (Battisti et al., 2010; Benedetti et al., 2010; Caruso et al., 2015; Leonard and Markey, 2008; Smith and Pearson, 2011; Van den Eede et al., 2012). MRSA CC398 is a zoonotic agent; those affected are primarily people who work with livestock (Liu et al., 2015) and have contact with animals. However, infections have also been reported in people who have had no contact with animals (Cuny et al., 2010; Köck et al., 2013; EFSA, 2009a; Pan et al., 2009; Soavi et al., 2010; Mammina et al., 2010; Lozano et al., 2011). In addition, Larsen et al. (2016), have recently suggested that foodborne transmission of LA-MRSA might occur, with meat implicated as a probable source. In this study, we

Table 4
Antimicrobial resistance profile of MRSA isolates from pigs.

		N. (%) OF RESISTANT STRAINS																			
SPA-TYPE/ SEQUENCE TYPE/ SCC*mec*	N. OF TESTED STRAINS	AK	AUG	AMP	KF	CTX	CF	DA	C	DXT	ENR	E	CN	K	OX	P	S	ST	TE	SXT	VA
ST398/t011/V	31	4 (13)	26 (84)	31 (100)	2 (6)	30 (97)	31 (100)	24 (77)	13 (42)	29 (94)	25 (81)	19 (61)	4 (13)	9 (29)	31 (100)	31 (100)	19 (61)	18 (58)	31 (100)	19 (61)	0 (0)
ST398/t034/V	28	1 (4)	12 (43)	28 (100)	1 (4)	28 (100)	28 (100)	25 (89)	0 (0)	22 (79)	19 (68)	20 (71)	0 (0)	5 (18)	28 (100)	28 (100)	22 (79)	13 (46)	27 (96)	13 (46)	0 (0)
ST398/t899/V	9	0 (0)	4 (44)	9 (100)	0 (0)	9 (100)	9 (100)	8 (89)	1 (11)	9 (100)	1 (11)	3 (33)	0 (0)	3 (33)	9 (100)	8 (89)	2 (22)	7 (78)	9 (100)	7 (78)	0 (0)
ST398/t1451/V	5	0 (0)	3 (60)	4 (80)	0 (0)	5 (100)	5 (100)	5 (100)	4 (80)	5 (100)	5 (100)	5 (100)	0 (0)	2 (40)	4 (80)	5 (100)	3 (60)	4 (80)	5 (100)	4 (80)	0 (0)
ST398/t1456/V	2	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)	2 (100)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	2 (100)	1 (50)	0 (0)	2 (100)	0 (0)	0 (0)
ST9/t4474/V	2	0 (0)	1 (50)	2 (100)	0 (0)	2 (100)	2 (100)	2 (100)	0 (0)	2 (100)	0 (0)	1 (50)	0 (0)	1 (50)	2 (100)	2 (100)	1 (50)	1 (50)	2 (100)	1 (50)	0 (0)
ST398/t588/V	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
ST398/t1793/V	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
ST97/t4795/V	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
ST97/t1730/V	1	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
ST97/t9301/V	1	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
ST1/t127/IVa	1	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)

Legend: AK, amikacin; AUG, amoxicillin/clavulanic acid; AMP, ampicillin; KF, cephalotin; CTX, cefotaxime; CF, cefoxitin; DA, clindamycin; C, chloramphenicol; DXT, doxycycline; ENR, enrofloxacin; E, erythromicin; CN, gentamicin; K, kanamicin; OX, oxacillin; P, penicillin; S, streptomycin; ST, sulfisoxazole; TE, tetracycline; SXT, trimetoprim-sulfamethoxazole; VA, vancomycin.

investigated the prevalence of MRSA in industrially reared swine in Southern Italy and in people who have contact with them. Our study shows the prevalence of MRSA in pigs (local and imported) to be high (59.1%), in agreement with data previously reported in literature (38–52%) (Battisti et al., 2010; EFSA, 2009b; EFSA, 2010; Normanno et al., 2015). MRSA has already been reported as more prevalent in fattening farms than in other kinds of farms, and this has been attributed to a greater exposure to external contamination due to the frequent introduction of new animals (Crombé et al., 2012; EFSA, 2009b; EFSA, 2010). Moreover, MRSA was significantly more prevalent in imported than in local pigs; this may be associated with long journeys during which imported pigs are confined in a limited space, as suggested by other authors (Broens et al., 2010).

Pig farmers are among the categories at highest risk for MRSA colonization (Armand-Lefevre et al., 2005; Huijsdens et al., 2006); reported prevalence ranges widely, from 15 to 37.8%. (Cui et al., 2009; Denis et al., 2009). Our survey included a large number of pig farmers and showed a high prevalence of colonization with MRSA (19.2%). The percentage of MRSA-positive farmers was significantly higher on fattening farms than on breeding farms. Thus, it appears that frequent and direct contact with the animals increases the risk of infection; in addition, the fact that some operators work on more than one fattening farm, increases the risk of being exposed to MRSA. On the whole, operators in MRSA-positive farms had a significantly higher risk to acquire the infection. Furthermore, our study found that MRSA prevalence was significantly higher in farmers (19.2%) than in abattoir workers (5.0%). A possible explanation for this finding is that farmers are constantly exposed to animals colonized with MRSA, while abattoir workers only have brief contact with the animals. It is known that at abattoir, exposure to MRSA in the air might play a major role for the spread of the organism from animals to abattoir workers (Gilbert et al., 2012) but, on the other hand, the food hygiene regulations state that food handlers (e.g. abattoir workers) must be trained in food hygiene, and must be protected against contamination (protective clothing, hygienic washing and drying of hands, cleansing and disinfection of equipment coming into contact with food, etc.) at all stages of production, processing and distribution. In our report, the prevalence of MRSA in abattoir workers appears lower than the one reported previously in southern Italy by Normanno et al., who conducted a survey on 113 abattoir workers in Italy reporting a 76% prevalence (Normanno et al., 2015). A possible explanation of these differences in prevalence could be due to the fact that the abattoir workers tested were employed in different steps of the slaughter process; indeed, it is demonstrated that working in the lairage, scalding and dehairing area, represents a major risk to contract the infection. (Gilbert et al., 2012).

Although MRSA ST398 is the most prevalent pig-associated genotype in several countries, including Italy, other genotypes of MRSA typically associated to animals (ST97, ST9) or to humans (ST1, ST5) have been detected. Our study identified 12 different genotypes from pigs and 7 from humans, with ST398 (t034, t011 and t899) representing the dominant genotype in both species. This finding agrees with other Italian and European surveys (Battisti et al., 2010; EFSA, 2009b; EFSA, 2010; Smith and Pearson, 2011). The 84.6% of MRSA strains isolated from humans were indistinguishable from those isolated from swine sampled in the same farms and/or abattoirs, suggesting that transmission might occur in both directions: human to animal and animal to human. An investigation of the phylogeny of CC398 showed that livestock-associated MRSA ST398 was originally a human methicillin-susceptible *S. aureus* (MSSA), which has adapted to livestock, losing genes associated with the potential to infect humans (such as the Pantone-Valentine Leucocidin coding genes and the immunological escape cluster coding genes) and acquiring some antimicrobial-resistance coding genes, such as the *mec*A and *tet* genes (Wulf and Voss, 2008). For this reason, LA-MRSA ST398, although resistant to several antimicrobials, is generally recognized as mildly virulent for humans. However, human exposure to livestock-associated MRSA ST398 poses a

Table 5
Antimicrobial resistance profile of MRSA isolates from humans.

N. OF RESISTANT STRAINS																				
SPA-TYPE/SEQUENCE TYPE/SCC*mec*	N OF TESTED STRAINS	AK	AUG	AMP	KF	CTX	CF	DA	C	DXT	ENR	E	CN	K	OX	P	S	ST	TE	SXT	VA
ST398/t034/V	10	0	6	10	1	9	10	9	1	8	4	7	0	3	10	9	7	4	9	4	0
ST398/t011/V	6	0	3	6	0	6	6	6	0	6	2	6	3	3	6	6	3	1	6	1	0
ST398/t899/V	6	0	4	6	0	6	6	6	1	6	2	4	1	0	6	6	3	4	6	4	0
ST9/t1939/V	1	0	0	1	0	1	1	1	0	1	1	1	0	0	1	1	0	1	1	1	0
ST97/t1730/V	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0
ST1/t127/V	1	0	1	1	0	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0
ST5/t002/N.D.	1	0	1	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	0	0

Legend: AK, amikacin; AUG, amoxicillin/clavulanic acid; AMP, ampicillin; KF, cephalotin; CTX, cefotaxime; CF, cefoxitin; DA, clindamycin; C, chloramphenicol; DXT, doxycycline; ENR, enrofloxacin; E, erythromicin; CN, gentamicin; K, kanamicin; OX, oxacillin; P, penicillin; S, streptomycin; ST, sulfisoxazole; TE, tetracycline; SXT, trimetoprim-sulfamethoxazole; VA, vancomycin.

potential risk for human health, since it might facilitate the re-adaptation of this clone to humans via potential reacquisition of genes associated with human pathogenicity (Alba et al., 2015; Price et al., 2012). In support of this hypothesis, Larsen has recently reported the case of a novel hybrid LA-MRSA CC9/CC398 genotype that has adapted to humans (Larsen et al., 2016). Human colonization by MRSA is a significant threat to public health, because human carriers can develop a clinical disease (Cohn and Middleton, 2010; Jordan et al., 2011) and spread MRSA to other people, particularly to sensitive populations e.g., infants, the elderly, and immunocompromised individuals. In addition, the carriage state of LA-MRSA could be persistent, leading to the spread of this resistant organism between humans (Bosch et al., 2014) in households and communities (van Cleef et al., 2015).

Resistant microbes can contaminate foods and food surfaces, potentially leading to human infection or food-borne intoxication. In this study, five of the human and swine isolates carried the genes encoding staphylococcal enterotoxins.

In line with other reports (Battisti et al., 2010; EFSA, 2009b; EFSA, 2010), our survey identified the Sequence Types ST1, ST9, and ST97, which were isolated in both swine and humans, and ST5, which was detected only in a human sample. MRSA ST1/t127 strains are typically associated with human infections and have caused serious infections of communities in the United States, Germany, and the United Kingdom (Köck (a) et al., 2013; Otter and French, 2008; Witte, 2009). In addition, these strains are among the most common clones isolated in European cases of invasive human MRSA infections (Monaco et al., 2013; Otter et al., 2009). This genotype has been isolated in different animal species, such as swine, cattle, horses, sheep and goats (Franco et al., 2011; Petinaki and Spiliopoulou, 2012; Pilla et al., 2012). The findings highlight the potential for livestock to serve as a reservoir for these strains (Battisti et al., 2010; Benedetti et al., 2010; Caruso et al., 2015; Parisi et al., 2016). A recent phylogenetic analysis of the CC1 (ST1) strains has shown a low relatedness between MRSA-CC1 in swine and humans, unlike the high relatedness reported between the cattle and human strains (Alba et al., 2015). Therefore, given their weak phylogenetic correlation and adaptability to humans, MRSA strains isolated from pigs appear less virulent for humans than cattle strains (Franco et al., 2011; Alba et al., 2015). ST97 is reported as the second most prevalent pig-associated MRSA lineage in the Italian pig industry, after ST398 (Battisti et al., 2010). MSSA ST97 is typically associated with cattle, but it is unclear whether the MRSA ST97 originated in cattle or has been transmitted as MSSA from pigs to cattle; it may have acquired resistance to antibiotics in intensive pig farms and returned to cattle in those areas with a high density of both pigs and cattle. Regardless of this clone's origin, the interspecies cattle to swine transmission or *vice versa* has suggested that *S. aureus* clones may also be transmitted to humans, and the animal origin of a MRSA ST97 clone capable of infecting humans has recently been demonstrated (Feltrin et al., 2015; Spoor et al., 2013). ST9 is the most prevalent LA-MRSA clone among pigs in most Asian countries. In China, ST9 MRSA has been isolated in food animals and animal-derived products (pork, chicken and raw milk), and has also been found in farm workers (Cui et al., 2009; Yan et al., 2016; Yang et al., 2016; Sun et al., 2015). MRSA ST9 lineage has also been reported in the USA and Europe, including Italian pig holdings (Battisti et al., 2010; EFSA, 2009b; EFSA, 2010; Sun et al., 2015). ST5 is a MRSA genotype typically associated with clinical infections in humans, and is among the most prevalent ones causing serious infections both in hospitals and in the community. Strains associated with hospital (HA-MRSA) and community (CA-MRSA) infections exhibit increased virulence compared to LA-MRSA ST5 isolates, which have a reduced capacity to cause severe disease in immunocompetent humans (Hau et al., 2015). In the present study, the isolation of MRSA strains with different genetic profiles in all the pig farms (fattening or breeding) surveyed was noteworthy. Similar findings were reported by Varhegghe et al., that reported a genetic diversity in LA-MRSA isolates from piglets along the pig production chain (Varhegghe et al., 2014). While more than one genotype was detected in both pigs and farm workers in pig fattening farms, different genotypes were isolated only in pigs in breeding farms. Other authors have already reported that the coexistence of strains belonging to different genetic lineages in the same farm may play a substantial role in the spread of virulence factors (Battisti et al., 2010). There are two conclusions to be made regarding the antimicrobial resistance profile of the MRSA strains isolated from humans and animals: i) both swine and human isolates had the characteristics of general multidrug resistance, as reported in other industrialized countries (Weese and van Duijkeren, 2010). The Armand-Lefevre study observed differences in susceptibility to antimicrobial molecules between strains displaying the same genotype, suggesting that the phenotypes are selected on-farm under the selective pressure due to the antimicrobial agent used (Armand-Lefevre et al., 2005). ii) Not all the LA-MRSA strains isolated were resistant to tetracycline, as previously reported in literature (Battisti et al., 2010; Normanno et al., 2015). The extensive use of tetracycline in pig farming has contributed to the selection of MRSA ST398 in this species (EFSA, 2009b; EFSA, 2010). However, the continuous evolution of this organism and the use of other classes of antimicrobials in the pig production chain, may have promoted the selection of strains that have modified their sensitivity pattern to antimicrobials.

Given these findings, further investigations are required to clarify the prevalence of these threatening strains and the mechanisms they use to spread.

In the context of “One Health”, improved animal welfare and reduced use of antimicrobials in farming, preventive measures to minimize levels of MRSA contamination in animals along the entire pig production chain, and improved food hygiene awareness in food operators and consumers would play a crucial role in reducing the transmission of LA-MRSA to humans.

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