



## Methicillin-resistant *Staphylococcus aureus* (MRSA) in slaughtered pigs and abattoir workers in Italy



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### ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen present in the hospital environment (HA-MRSA), in the community (CA-MRSA) and in livestock, including pigs (LA-MRSA). MRSA may enter the human food chain during slaughtering and may infect humans coming into direct contact with pigs or pork products. This study aimed to determine the prevalence and characteristics of MRSA isolated from pigs and workers at industrial abattoirs in southern Italy. A total of 215 pig nasal swabs were screened for the presence of MRSA using PCR. An MRSA isolate was detected from each *mecA/nuc* PCR-positive sample and characterized by *spa*-typing, Multi-Locus Sequence Typing, SCC-*mec* and Panton-Valentine Leukocidin (PVL), and also tested for the production of staphylococcal enterotoxins (SEs).

Eighty-one MRSA isolates (37.6%) were obtained from the 215 pig nasal swabs; 37 of these isolates were further characterized, and showed 18 different *spa*-types and 8 different STs. The most frequently recovered STs were ST398 (CC398-t034, t011, t899, t1939 – 43.2%) followed by ST8 (CC8-t008, t064, t2953, t5270 – 24.3%) and ST1 (CC1-t127, t174, t2207 – 10.8%).

Nine MRSA isolates were obtained from the 113 human swabs; the isolates showed 5 different *spa*-types and 5 different STs, including the novel ST2794 (t159). The most representative STs recovered were ST1 (CC1-t127) and ST398 (CC398-t034) (33.3%). None of the MRSA isolates showed the ability to produce SEs and PVL and all resulted resistant to two or more classes of antimicrobials. This study shows the great genetic diversity of MRSA strains in slaughtered pigs and in abattoir employees in Italy, and clearly demonstrates the need for improved hygiene standards to reduce the risk of occupational and food-borne infection linked to the handling/consumption of raw pork containing MRSA.

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### 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are well-known worldwide as causing hospitalizations and deaths (de Lencastre et al., 2007). Mortality rates for invasive blood-stream and pneumonic infections are very high (Klevens et al., 2007), and severe wound infections may also occur (Köck et al., 2010). MRSA infections originated in hospitals (Hospital Acquired, HA-MRSA) (Livermore, 2000) and then emerged into the community

(Community Acquired, CA-MRSA) (Naimi et al., 2003; Kluytmans-Vandenbergh, 2006). A zoonotic MRSA clade associated with farm animals (Livestock-Associated, LA-MRSA) has been identified as a cause of human infections; Voss (Voss et al., 2005) provided the first report of MRSA ST398 in pigs and pig farmers in the Netherlands. Subsequent reports have demonstrated that this clade is also widespread in Europe and other industrialized countries, including the USA, Canada, China and Korea (Khanna et al., 2008; Cui et al., 2009; Lim et al., 2012). This clade is non-typeable by PFGE using *Sma*I, and was subsequently found to belong to the Multi-Locus Sequence Type (ST) ST398 and Clonal Complex (CC) 398 (van Loo et al., 2007), that includes livestock-associated and livestock-independent MRSA strains (Stegger et al., 2013). ST398

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was also found to colonize farmers, veterinarians, and people in contact with farm animals (Petinaki and Spiliopoulou, 2012). However, LA-MRSA was also found in people without livestock contact (Lekkerkerk et al., 2012). These findings clearly demonstrate that MRSA CC398 and others (e.g. CC5, CC9, CC97) have a zoonotic role linked to food-producing animals such as pigs, cattle and poultry (de Neeling et al., 2007; Huijsdens et al., 2006; Leonard and Markey, 2008; Voss et al., 2005; Wulf et al., 2006; Smith et al., 2008; Köck et al., 2013). When MRSA-carrying animals are slaughtered, MRSA may spread to carcasses, to the environment and to abattoir workers. In addition, if carcasses and meat are contaminated, MRSA can enter the human food chain (Kluytmans, 2010). Several papers have reported MRSA contamination of food-stuffs, highlighting the potential risk of transmission to humans via food handling/consumption (Normanno et al., 2007; De Boer et al., 2009; EFSA, 2009b; van Loo et al., 2007; Virgin et al., 2009; Agersø et al., 2012). Every year, about 14 million pigs from local farms and from other European countries are slaughtered in Italy, and over 3000 people work in the pork production chain (slaughtering and cutting) (ISTAT, 2013).

As far as we are aware, despite high levels of pork consumption, no reports are available on the prevalence and the characteristics of MRSA isolated from slaughtered pigs – regardless of their origin – nor about rates of MRSA colonization in Italian abattoir workers. This study aims to provide information about the prevalence and characteristics of MRSA isolates from abattoir workers and slaughtered pigs, in order to create the basis for further study on defining the risks of occupational and food-borne transmission linked to the handling or consumption of pork products in Italy.

## 2. Materials and methods

### 2.1. Sampling

A total of 328 samples were taken at two industrial abattoirs in southern Italy: 215 from pigs and 113 from abattoir workers. Over one year, we convenience sampled 215 slaughtered pigs by taking one nasal swab from each pig immediately after the stunning/lairage stage (Amies agar transport swabs, Copan, Brescia, Italy). Each month, we sampled approximately 10 carcasses per slaughterhouse. The abattoirs selected for the study slaughtered about 3000 pigs per week, a large proportion of the total number of pigs slaughtered in southern Italy. The pigs examined came from Italy, and were also imported from Belgium and Spain.

Nasal swabs were taken from the anterior nares of 113 abattoir workers whose written consent had been obtained in advance. The workers included in the study were those who slaughter and cut the carcasses.

Swabs were chilled and transported to the laboratory immediately after collection, and then analysed within 10 h.

Our study was approved by the Medical Ethical Committee of the Bari Medical Authority – ASL BA (ID 244, 321, 921).

### 2.2. Detection of nuc and mecA genes

Each swab was placed in 5 ml of Mueller-Hinton Broth (MHB, Liofilchem, Teramo, Italy) supplemented by 6.5% NaCl (Sigma-Aldrich, St. Louis, Mo, USA), and incubated at 37 °C for 16–20 h in aerobic conditions (de Boer et al., 2009). The Genomic Prep DNA isolation Kit (Amersham Pharmacia Biotech, New York, USA) was used to extract bacterial DNA from 1 ml of each culture broth, following the manufacturer's instructions. The extracts were subjected to a duplex-PCR protocol for the detection of *mecA* and *nuc* genes (Virgin et al., 2009). A methicillin-susceptible *S. aureus* strain

(ATCC 29213) was used as a negative control, and MRSA strain (ATCC 33591) as a positive control.

### 2.3. Isolation and identification of MRSA

The *nuc/mecA* positive samples were seeded on two plates of MRSA ID (bioMerieux, France) (Moodley et al., 2008; de Boer et al., 2009). After incubation at 37 °C for 24 h in aerobic conditions, 5 colonies for each positive sample showing the phenotypic characteristics of MRSA were seeded on Trypton Soy Agar (Liofilchem, Teramo, Italy). After further incubation for 24 h at 37 °C in aerobic conditions, these were tested to confirm identification using the PCR protocol reported above.

### 2.4. MRSA characterization

A randomly selected number ( $n = 37$ ) of strains isolated from pigs and all the MRSA strains ( $n = 9$ ) isolated from the abattoir workers were characterized in order to assess the *spa*-type, Sequence Type, Clonal Complex, Staphylococcal Cassette Chromosome-*mec*, Panton-Valentine Leukocidin, staphylococcal enterotoxin(s) production and the antimicrobial resistance pattern.

### 2.5. Spa-typing

The *x* region of the *spa* gene was amplified by PCR using primers spa-1113f (5' TAA AGA CGA TCC TTC GGT GAG C 3') and spa-1514r (5' CAG CAG TAG TGC CGT TTG CTT 3') (Strommenger et al., 2006). DNA sequences were obtained using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, Calif.) and BigDye 3.1 Ready reaction mix (Applied Biosystems) according to the manufacturer's instructions. BioNumerics 7.1 (Applied Maths, Belgium) software was used to determine *spa* types.

### 2.6. Multi Locus Sequence Typing

The PCR amplifications were performed using the primers described elsewhere (Enright et al., 2000), and sequence reactions were carried out as described above. Sequences were imported and assembled using BioNumerics 7.1 software (Applied Maths). Alleles and ST were assigned by submitting the DNA sequences to the *Staphylococcus* MLST database (<http://saureus.mlst.net/>). Strains were grouped into clonal complexes, defined as groups of profiles differing by no more than one gene from at least one other profile of the group as defined in the MLST database.

### 2.7. SCC-mec characterization

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

### 2.8. PVL detection

MRSA selected strains were tested by PCR for *lukS-PV* and *lukF-PV* encoding Panton-Valentine Leukocidin (PVL), as described elsewhere (Kocsis et al., 2009).

### 2.9. Detection of staphylococcal enterotoxins (SEs)

The selected strains were tested to detect SEA, SEB, SEC and SED by Reverse Passive Latex Agglutination (RPLA), using the SET RPLA kit (Oxoid) according to the manufacturer's instructions. In addition, MRSA strains were tested by PCR for *sea* to *sed* encoding staphylococcal enterotoxins SEA to SED (Monday and Bohach, 1999).

### 2.10. Antimicrobial susceptibility testing of MRSA

MRSA isolates were tested for susceptibility to a panel of 10 antimicrobial agents using the disc agar diffusion method on Mueller-Hinton agar, following the CLSI guidelines (CLSI, 2012). The antibiotic discs (antibiotic concentration in µg) from Liofilchem (Liofilchem s.r.l., Roseto d. A., Italy) are as follows: ampicillin (10), cephalotin (30), cefoxitin (30), clindamycin (2), erythromycin (15), kanamicin (30), oxacillin (1), penicillin (10), tetracycline (30) and vancomycin (30). The results were recorded after 24 h of incubation at 37 °C and interpreted according to charts supplied with the discs.

### 2.11. Statistical analysis

StatView 5.0 software (SAS Institute Inc.) was used for statistical analysis and the chi-square test was performed to analyze nominal variables.

## 3. Results

### 3.1. Slaughtered pigs

We isolated 81 MRSA strains (37.6%) from the 215 pig swabs, and identified 18 different spa-types and 8 different STs from characterization of the 37 selected isolates.

The most frequently recovered ST (16 isolates, 43.2%) was ST398 (CC398-t034, t011, t899, t1939) followed by ST8 (CC8-t008, t064, t2953, t5270) (9 isolates 24.3%), ST1 (CC1-t127, t174, t2207) (4 isolates, 10.8%) (Table 1).

The most frequently recovered spa-type was t034 (10 isolates, 27%), followed by t064 (4 isolates, 10.8%), t011 and t2953 (3 isolates, 8.1%), t127, t528, t988, t2136 (2 isolates, 5.4%) and t008, t012, t084, t085, t093, t174, t2207, t5270, and t1939 (1 isolate, 2.7%) (Table 1).

All MRSA isolates from pigs were unable to synthesize the investigated SEs and resulted lukS-PV and lukF-PV negative (Tables 1 and 2). All isolates harboured the SCC-mec type IV (48.6%) or V (45.9%). In two isolates (5.4%), both CC9/ST2136/t337, the SCC-mec resulted not detected (Table 1).

All isolates from pigs were multidrug resistant. In addition to resistance to beta-lactams, the isolates showed various antimicrobial resistance patterns (Table 1); two common antimicrobial resistance profiles were clindamycin–kanamycin–tetracycline, and

erythromycin–kanamycin–tetracycline. Resistance to clindamycin, erythromycin, kanamycin, and tetracycline was detected in two isolates (Table 1). All isolates were resistant to tetracycline but susceptible to vancomycin.

### 3.2. Abattoir workers

Nine MRSA strains were isolated from the 113 human swabs. The isolates presented 5 different spa types and 5 different STs. The novel ST2794 was identified. The most frequently recovered STs were ST1 (CC1-t127) and ST398 (3 isolates, 33.3%) (CC398-t034), followed by ST8 (CC8-t008), ST15 (CC15-t084) and the novel ST2794 (CC1-t159). CC1 and CC398 were the most frequently recovered CCs, followed by CC8 and CC84 (Table 2).

In addition to being resistant to beta-lactams, the human isolates were also highly resistant to erythromycin and clindamycin (Table 2). All isolates were resistant to tetracycline but susceptible to vancomycin.

All human isolates were unable to synthesize the investigated SEs and resulted PVL negative; all harboured the SCC-mec type IV (66.6%) or V (33.3%) (Tables 1 and 2).

No statistically significant differences were found regarding the origin of the samples, and MRSA strains were isolated during the year without any statistical difference observed between months.

## 4. Discussion

Human infections caused by MRSA are a leading cause of morbidity and mortality in industrialized countries. In addition to the traditional routes of MRSA infection, it has recently been demonstrated that direct transmission to humans takes place via contact with farm animals (Wendlandt et al., 2013). Another suggested infection risk is created by the handling of animal food products, such as raw meat (EFSA, 2009b; Köck et al., 2013). Many studies on the spread of MRSA in farm animals and their carcasses have focused on pigs, which are currently the most important reservoir of MRSA ST398 and other livestock-associated clones (Overesch et al., 2011; Gómez-Sanz et al., 2010). MRSA contaminations have frequently been recorded in pigs slaughtered in Germany, the Netherlands, Belgium, Denmark, Canada, the USA and Singapore (de Neeling et al., 2007; Sergio et al., 2007). In addition, two large Italian surveys showed that 38%–52% of pigs in

**Table 1**  
Characteristics of MRSA isolated from pigs at slaughter.<sup>a</sup>

Number of isolates	CC	ST	Spa-type	Repeat succession	SCC-mec	lukS-PV lukF-PV	SEA to SED (sea to sed)	Resistance profile to non beta-lactam antibiotics
2	1	1	t127	07-23-21-16-34-33-13	IV	–	– (–)	CLI–ERY–KAN–TET
1	1	1	t2207	07-33-13	IV	–	– (–)	CLI–KAN–TET
1	1	1	t174	14-21-16-34-33-13	IV	–	– (–)	CLI–KAN–TET
4	8	8	t064	11-19-12-05-17-34-24-34-22-25	IV	–	– (–)	ERY–KAN–TET
1	8	8	t5270	11-12-21-17-34-24-34-22-25-25-25	IV	–	– (–)	ERY–KAN–TET
3	8	8	t2953	11-12-21-17-34-24-34-22-25-25	IV	–	– (–)	ERY–KAN–TET
1	8	8	t008	11-19-12-21-17-34-24-34-22-25	IV	–	– (–)	ERY–TET
2	9	2136	t337	07-16-23-23-02-12-23-02-34	N.D.	–	– (–)	TET
1	15	15	t084	07-23-12-34-34-12-12-23-02-12-23	IV	–	– (–)	TET
1	15	15	t085	07-23-12-34-34-12-23-02-12-23	IV	–	– (–)	TET
1	30	30	t093	15-12-12-16-02-16-02-25-17-24-24	V	–	– (–)	ERY–TET
1	30	30	t012	15-12-16-02-16-02-25-17-24-24	IV	–	– (–)	ERY–TET
2	59	59	t528	04	IV	–	– (–)	ERY–TET
10	398	398	t034	08-16-02-25-02-25-34-24-25	V	–	– (–)	CLI–ERY–TET
3	398	398	t011	08-16-02-25-34-24-25	V	–	– (–)	CLI–ERY–TET
2	398	398	t899	07-16-23-02-34	V	–	– (–)	CLI–TET
1	398	398	t1939	07-23-02-34	V	–	– (–)	CLI–TET

ND = not detected.

ERY = erythromycin, CLI = clindamycin, TET = tetracycline; KAN = kanamycin.

<sup>a</sup> Isolates showing the same characteristics are grouped in a single row.

**Table 2**Characteristics of MRSA isolated from abattoir workers.<sup>a</sup>

Number of isolates	CC	ST	Spa-type	Repeat succession	SCC-mec	<i>lukS-PV</i> <i>lukF-PV</i>	SEA to SED (sea to sed)	Resistance profile to non beta-lactam antibiotics
3		1	1	t127	07-23-21-16-34-33-13	IV	–	– (–)
1		1	2794	t159	14-44-13-12-17-17-23-18-17	IV	–	– (–)
1		8	8	t008	11-19-12-21-17-34-24-34-22-25	IV	–	– (–)
1		15	15	t084	07-23-12-34-34-12-12-23-02-12-23	IV	–	– (–)
3		398	398	t034	08-16-02-25-02-25-34-24-25	V	–	– (–)

ND = not detected.

ERY = erythromycin, CLI = clindamycin, TET = tetracycline; KAN = kanamycin.

<sup>a</sup> Isolates showing the same characteristics are grouped in a single row.

investigated herds were MRSA carriers (EFSA, 2009a; Battisti et al., 2010). On this basis, we studied the prevalence and the characteristics of MRSA in slaughtered pigs and in workers at two industrial abattoirs in Southern Italy. In our study, as expected, the most frequently recovered ST was ST398, followed by ST8. However, Molla observed that CC398 was the second (12/50) most common CC detected in a study on the epidemiology and the genetic characteristics of MRSA from pigs (Molla et al., 2012). ST8 (t008) is already known as a human MRSA strain, but has also been detected in pigs in Norway (Sunde et al., 2011), and was found in pork samples in the USA, during two surveys on the presence of MRSA in retail meat in Iowa (Hanson et al., 2011), and in Iowa, Minnesota and New Jersey (O'Brien et al., 2012). Other STs recovered were ST1, ST15, ST30, ST59, and ST 2136. Two strains among the 18 spa-types recovered from pigs were identified as t127 (ST1). Battisti was the first to report the presence in Italian pigs of the spa-type t127 (ST1), known as a human MRSA lineage; this lineage has been associated with serious human infections in the USA and in Germany (Battisti et al., 2010; CDC, 1999; Cuny et al., 2008). In Denmark, a single t127 was detected out of 101 MRSA isolates from slaughtered pigs, accounting for 0.1% of the total number of pigs tested (n = 789) (Agersø et al., 2012). It is known that t127/ST1 can be assigned to two genetically different clusters (porcine and human), thus this strain may be considered another LA-MRSA lineage (Franco et al., 2011). CC398 and CC8 were the most representative CCs, followed by CC1, CC9, CC15, CC30 and CC59. According to Battisti, the presence of different lineages of different origin (human and animal) clearly demonstrates that pigs may acquire and spread MRSA belonging to CCs other than CC398, acting as an important reservoir of MRSA infection (Battisti et al., 2010). The presence of MRSA in the nasal cavities could mean that it spreads onto the surface of the carcasses during cutting, and then onto the meat. There are actually several reports on the prevalence of MRSA in various kinds of meats from Japan, Korea, Europe, the USA and other countries (Kitai et al., 2005; Know et al., 2006; de Boer et al., 2009; O'Brien et al., 2012). The presence of MRSA in pig carcasses for human consumption is a public health issue, and the emergence of MRSA ST398 infections in humans has been well documented in several European countries (Witte et al., 2007). Italy has a high prevalence of MRSA infection compared with other European countries, and the majority of MRSA belong to six major clones: ST8-MRSA-I, ST247-MRSA-IA, ST239-MRSA-IIIA, ST228-MRSA-I, ST247-MRSA-I/IA and ST22-MRSA-IV and several minor clones. ST228 is the most common HA-MRSA clone (Campanile et al., 2009).

However, there are three reported cases of invasive MRSA ST398 infection in Italy: a pig-farm worker with cellulitis and pyomyositis (Pan et al., 2009), a dairy cattle farmer with necrotizing fascicolitis (Soavi et al., 2010), and a patient with ventilator-associated pneumonia (Mammìna et al., 2010). A recent study by Monaco reports that the overall rate of ST398 colonization in an area of Italy with a high density of pig farming was 0.56%, a percentage in the range

(<0.01–1.2%) described in the Netherlands and Germany, regarding the non-exposed healthy population. However, the authors concluded that this clade rarely produce infections in the studied area (Monaco et al., 2013). A recent paper by Köck has analysed 14,036 MRSA isolates from human clinical samples and screening specimens, finding 23% of CC398 in screening samples. The author states that rigorous surveillance of MRSA CC398 and other putative LA-MRSA (CC5, CC9, CC30, CC97) is necessary because they are a major cause of human infection in Germany (Köck et al., 2013).

Regarding the risk of food-borne intoxication, many types of staphylococcal enterotoxins (SEs) are reported, but it is well known that the most important serological types are SEA, SEB, SED, and SEC. In our survey, no MRSA isolates from pigs were enterotoxigenic. This finding agrees with those reported by other researchers (Kluytmans, 2010), and indicates that the risk of foodborne intoxication linked to consumption of MRSA-contaminated meat is quite limited. The presence of MRSA in pigs is also a potential professional hazard for those working in the meat production chain (i.e. farmers, transporters, butchers, and veterinarians). It is known that people working several hours per week in direct contact with MRSA-positive animals are exposed to a high risk of nasal colonization (van Loo et al., 2007; Moodley et al., 2008; Voss et al., 2005; Denis et al., 2009; Witte et al., 2007). The general population shows a high prevalence (about 30%) of *S. aureus* nasal colonization, whereas MRSA nasal colonization levels appear low (0.7–1.5%), depending on the geographical area (Gorwitz et al., 2008; Wertheim et al., 2004; Munckhof et al., 2009). Human colonization implies that carriers become a bacterial reservoir and may transfer the infection to others, or contaminate foods and food surfaces during handling. Moreover, subclinical carriage of MRSA by humans is considered a risk factor for subsequent occurrence of clinical disease, increasing this risk by up to 10-fold (Cohn and Middleton, 2010; Jordan et al., 2011). Many studies have investigated MRSA nasal colonization among workers in contact with animals, especially veterinarians and farmers (van Cleef et al., 2014), but little data is available about its prevalence among abattoir workers, although MRSA from food-producing animals can be transferred to the abattoir environment and thus constitute a source of contamination for abattoir workers (Wendlandt et al., 2013). In our survey, the anterior nares of 9 out of 113 investigated workers were colonised by MRSA. The most prevalent STs recovered were ST1 (t127) and ST398 (t034), followed by ST8 (t008) and ST15 (t084). Using our techniques, these isolates appear indistinguishable from those isolated from pigs, in our survey. In addition, we detected one ST2794 (t159) strain that has never been described before. CC1 and CC398 were the most frequently recovered CCs. These findings are in contrast with those of Huber (Huber et al., 2010) who detected no MRSA in 179 abattoir workers in Switzerland, and with those of Cui (Cui et al., 2009), who reported no MRSA in 107 abattoir workers in China. Mulders found 26/466 (5.6%) MRSA-positive workers at a chicken abattoir, and concludes

that there is an increased risk of MRSA carriage in personnel working in this kind of abattoir (Mulders et al., 2010). On the other hand, van Cleef reported a prevalence of 5.6% of nasal carriage in 195 workers at a pig abattoir in the Netherlands, which was higher than the prevalence of 0.1% among the general population of the country. All the MRSA isolates belonged to the CC398 livestock-associated clade (van Cleef et al., 2010). Similar results were reported for the Netherlands by Gilbert, who found that 11 out of 341 pig slaughterhouse workers (3.2%) were nasal carriers of LA-MRSA (Gilbert et al., 2012). In a Spanish study on the prevalence of MRSA in pigs and pig workers, the authors found that as many as 14.3% of abattoir workers were nasal MRSA carriers (Morcillo et al., 2012).

Our isolates from both pigs and humans harboured two SCC-mec types, the SCC-mec IV and SCC-mec V. This is a common finding among LA-MRSA and CA-MRSA (Cohn and Middleton, 2010; Vanderhaeghen et al., 2010). Panton-Valentine Leukocidin (PVL) is a cytotoxin that causes leukocyte destruction and tissue necrosis, severe skin and soft tissue infections and highly lethal necrotizing pneumonia. This toxin is typically associated with certain CA-MRSA strains but generally lacking in LA-MRSA (Vanderhaeghen et al., 2010). In our survey, all the isolates tested negative for PVL and this agrees with other studies on the characterization of MRSA from pigs and humans (Battisti et al., 2010; Sunde et al., 2011).

Antimicrobial resistance has increased worldwide in human bacterial pathogens and in zoonotic agents and this may compromise the effective treatment of infections in humans. Multidrug resistance was prevalent in our MRSA isolates, and isolates in both groups (pigs and abattoir workers) displayed resistance to two or more classes of antimicrobials, but were fully susceptible to vancomycin. These findings are in agreement with other reports of high levels of antimicrobial resistance observed in MRSA isolates from pigs, their meat and humans (Battisti et al., 2010; Jackson et al., 2013). As expected all the pig isolates were resistant to tetracycline. This finding is well documented (particularly for ST398) and seems attributable to the large-scale use of this drug in pig farming (de Neeling et al., 2007). Finally, it is known that MRSA prevalence and types differ according to the geographical area and the density of the food-producing animal population. The pigs used in our survey originated from different farms and countries, and this could be the reason for the wide heterogeneity of the MRSA strains we found.

The presence of MRSA in carcasses intended for human consumption is a potential health hazard, and it must be controlled by implementing staff training on issues of biosecurity and food hygiene, throughout the meat chain from primary production to retail outlets. As far as we know, this is the first report documenting the prevalence and characteristics of MRSA in slaughtered pigs and in abattoir workers in Italy.

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## References

- Agersø, Y., Hasman, H., Frank, L.M., Aarestrup, F.M., 2012. Study of methicillin-resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. *Vet. Microbiol.* 157, 246–250.
- Battisti, A., Franco, A., Merialdi, G., Hasman, H., Iurescia, M., Lorenzetti, R., Feltrin, F., Zini, M., Aarestrup, F.M., 2010. Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Vet. Microbiol.* 142, 361–366.
- Campanile, F., Bongiorno, D., Borbone, S., Stefani, S., 2009. Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Italy. *Ann. Clin. Microbiol. Antimicrob.* 8, 22. Available from: <http://www.ann-clinmicrob.com/content/8/1/22>.
- Centers for Disease Control Prevention, 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*-Minnesota and North Dakota, 1997–1999. *JAMA* 282, 1123–1125.
- Clinical and Laboratory Standards Institute, 2012. Performance Standards for Antimicrobial Susceptibility Testing, 22nd Informational Supplement. M100-S22.
- Cohn, L.A., Middleton, J.R.A., 2010. Veterinary perspective of methicillin-resistant staphylococci. *J. Vet. Emerg. Crit. Care* 20 (1), 31–45.
- Cui, S., Li, J., Hu, C., Jin, S., Li, F., Guo, Y., Ran, L., Ma, Y., 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. *J. Antimicrob. Chemother.* 64, 680–683.
- Cuny, C., Strommenger, B., Witte, W., Stanek, C., 2008. Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. *Mary Ann Liebert, Inc.*, publishers, NY. *Microb. Drug Resist.* 14, 307–310.
- de Boer, E., Zwartkruis-Nahuis, J.T.M., Wit, B., Huijsdens, X.W., de Neeling, A.J., Bosch, T., van Oosterom, R.A.A., Vila, A., Heuvelink, A.E., 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int. J. Food Microbiol.* 134, 52–56.
- de Lencastre, Oliveira, D., Tomasz, A., 2007. Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. *Curr. Opin. Microbiol.* 10, 428–435.
- de Neeling, A.J., van den Broek, M.J., Spalburg, E.C., van Santen-Verheuel, M.G., Dam-Deisz, W.D., Boshuizen, H.C., van de Giessen, A.W., van Duijkeren, E., Huijsdens, X.W., 2007. High prevalence of methicillin-resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* 122, 366–372.
- Denis, O., Suetens, C., Hallin, M., Catry, B., Ramboer, I., Dispas, M., Willem, G., Gordts, B., Butaye, P., Struelens, M.J., 2009. Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerg. Infect. Dis.* 15 (7), 1098–1101.
- EFSA, 2009a. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. Part A: MRSA prevalence estimates. *EFSA J.* 7 (11), 1376.
- EFSA, 2009b. Scientific opinion of the panel on biological hazards on a request from the European commission on assessment of the public health significance of methicillin-resistant *Staphylococcus aureus* (MRSA) in animal and foods. *EFSA J.* 993, 1–73.
- Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol.* 38, 1008–1015.
- Franco, A., Hasman, H., Iurescia, M., Lorenzetti, R., Stegger, M., Pantosti, A., Feltrin, F., Ianzano, A., Porroero, M.C., Liapi, M., Battisti, A., 2011. Molecular characterization of spa-type t127, sequence type 1 methicillin-resistant *Staphylococcus aureus* from pigs. *J. Antimicrob. Chemother.* 66, 1231–1235.
- Gilbert, M.J., Bos, M.E.H., Duim, B., Urlings, B.A.P., Heres, L., Wagenaar, J.A., Heederik, D.J.J., 2012. Livestock-associated MRSA ST398 carriage in pig slaughterhouse workers related to quantitative environmental exposure. *Occup. Environ. Med.* 69, 472–478.
- Gómez-Sanz, E., Torres, C., Lozano, C., Fernández-Pérez, R., Aspiroz, C., Ruiz-Larrea, F., Zarazaga, M., 2010. Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. *Foodborne Pathog. Dis.* 7 (10), 1269–1277.
- Gorwitz, R.J., Kruszon-Mora, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J., 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in United States, 2001–2004. *J. Infect. Dis.* 197, 1226–1234.
- Hanson, B.M., Dressler, A.E., Harper, A.L., Scheibel, R.P., Wardyn, S.E., Roberts, L.K., Kroeger, J.S., Smith, T.C., 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J. Infect. Public Health* 4 (4), 196–74.
- Huber, H., Koller, S., Giezendanner, N., Stephan, R., Zweifel, C., 2010. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Euro Surveill.* 15 (16), pii:19542.
- Huijsdens, X.W., van Dijke, B.J., Spalburg, E., van Santen-Verheuel, M.G., Heck, M.E., Pluister, G.N., Voss, A., Wannet, W.J., de Neeling, A.J., 2006. Community-acquired MRSA and pig-farming. *Ann. Clin. Microbiol. Antimicrob.* 10 (5), 26.
- ISTAT, 2013. Annuario Statistico Italiano. [www.istat.it](http://www.istat.it).
- Jackson, C.R., Davis, J.A., Barrett, J.B., 2013. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. *J. Clin. Microbiol.* 51 (4), 1199–1207.
- Jordan, D., Simon, J., Fury, S., Moss, S., Giffard, P., Maiwald, M., Southwell, P., Barton, M.D., Axon, J.E., Morris, S.G., Trott, D.J., 2011. Carriage of methicillin-resistant *Staphylococcus aureus* by veterinarians in Australia. *Aust. Vet. J.* 89 (5), 152–159.
- Khanna, T., Friendship, R., Dewey, C., Weese, J.S., 2008. Methicillin-resistant *Staphylococcus aureus* colonization in pigs and in pig farm farmers. *Vet. Microbiol.* 128, 298–303.
- Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T., Kitagawa, H., 2005. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *J. Vet. Med.* 67, 107–110.
- Klevens, R.M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L.H., Lynfield, R., Dumyati, G., Townes, J.M., Craig, A.S., Zell, E.R., Fosheim, G.E., McDougal, L.K., Carey, R.B., Fridkin, S.K., 2007. Active Bacterial Core surveillance

- (ABCs) MRSA investigators. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *J. Am. Med. Assoc.* 298 (15), 1763–1771.
- Köck, R., Becker, K., Cookson, B., van Gemert-Pijnen, J., Harbart, S., Kluytmans, J., Mielke, M., Peters, G., Skov, R.L., Struelens, M.J., Tacconelli, E., Navarro Torné, A., Witte, W., Friedrich, A.W., 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenge in Europe. *Euro Surveill.* 15 (42), pii:19694.
- Köck, R., Schaumburg, F., Mellman, A., Köksal, M., Jurke, A., Beker, K., Friedric, A.W., 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as cause of human infection and colonization in Germany. *PLoS One* 8 (2) e55040, 1–4.
- Kocsis, E., Lagler, H., Pesti, N., Stich, K., Kristóf, K., Nagy, K., Hermann, P., Komka, K., Cekovska, Z., Graninger, W., Rozgonyi, F., 2009. Comparison of Austrian, Hungarian and Macedonian methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains in relation to prevalence of cytotoxin genes. *Microb. Pathog.* 46 (6), 328–336.
- Kluytmans, J.A., 2010. Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? *Clin. Microbiol. Infect.* 16 (1), 11–15.
- Kluytmans-Vandenbergh, M.F., Kluytmans, J.A., 2006. Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. *Clin. Microbiol. Infect.* 12 (1), 9–15.
- Knou, N.H., Park, K.T., Jung, W.K., Youn, H.Y., Lee, Y., Kim, S.H., Bae, W., Lim, J.Y., Kim, J.M., Hong, S.K., Park, Y.H., 2006. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. *Vet. Microbiol.* 117, 304–312.
- Lekkerkerk, W.S., van de Sande-Bruinsma, N., van der Sande, M.A., Tjon-A-Tsien, A., Groenheide, A., Haenen, A., Timen, A., van den Broek, P.J., van Wamel, W.J., de Neeling, A.J., Richardus, J.H., Verbrugh, H.A., Vos, M.C., 2012. Emergence of MRSA of unknown origin in the Netherlands. *Clin. Microbiol. Infect.* 18 (7), 656–661.
- Leonard, F.C., Markey, B.K., 2008. Methicillin-resistant *Staphylococcus aureus* in animals: a review. *Vet. J.* 175, 27–36.
- Lim, S.K., Nam, H.M., Jang, G.C., Lee, H.S., Junk, S.C., 2012. The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. *Vet. Microbiol.* 155, 88–92.
- Livermore, D.M., 2000. Antibiotic resistance in staphylococci. *Int. J. Antimicrob. Agents* 16, 3–10.
- Mammìna, C., Calà, C., Plano, M.R.A., Bonura, C., Vella, A., Monastero, R., Palma, D.M., 2010. Ventilator-associated pneumonia and MRSA ST 398, Italy. *Emerg. Infect. Dis.* 16 (4), 730–731.
- Molla, B., Byrne, M., Abley, M., Mathews, J., Jackson, C.R., Fedorka-Cray, P., Sreevatsan, S., Wang, P., Gebreyes, W.A., 2012. Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. *J. Clin. Microbiol.* 50 (11), 3687–3693.
- Monaco, M., Pedroni, P., Sanchini, A., Bonomini, A., Indelicato, A., Pantosti, A., 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* responsible for human colonization and infection in an area of Italy with high density of pig farming. *BMC Infect. Dis.* 13, 258. Available from: <http://www.biomedcentral.com/1471-2334/13/258>.
- Monday, S.R., Bohach, G.A., 1999. Use of multiplex PCR to detect classical and new described pyrogenic toxin genes in staphylococcal isolates. *J. Clin. Microbiol.* 37 (10), 3411–3414.
- Moedley, A., Nightingale, E.C., Stegger, M., Nielsen, S.S., Skov, R.L., Guardabassi, L., 2008. High risk for nasal carriage of methicillin-resistant *Staphylococcus aureus* among Danish veterinary practitioners. *Scand. J. Work. Environ. Health* 34 (2), 151–157.
- Morcillo, A., Castro, B., Rodriguez-Alvarez, C., González, J.C., Sierra, A., Montesinos, M.I., Abreu, R., Arias, A., 2012. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in pigs and pig workers in Tenerife, Spain. *Foodborne Pathog. Dis.* 9, 3.
- Mulders, M.N., Haenen, A.P.J., Geenen, P.L., Vesseur, P.C., Poldervaart, E.S., Bosch, T., Huijsdens, X.W., Hengeveld, P.D., Dam-Deisz, W.D.C., Graat, E.A.M., Mevius, D., Voss, A., Van De Giessen, A.W., 2010. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. *Epidemiol. Infect.* 138, 743–755.
- Munckhof, W.J., Nimmo, G.R., Schooneveldt, J.M., Schlebusch, S., Stephens, A.J., Williams, G., Huygens, F., Giffard, P., 2009. Nasal carriage of *Staphylococcus aureus*, including community-associated methicillin-resistant strains, in Queensland adults. *Clin. Microbiol. Infect.* 15, 149–155.
- Naimi, T.S., LeDell, K.H., Como-Sabetti, K., Borchardt, S.M., Boxrud, D.J., Etienne, J., 2003. Comparison of community and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *J. Am. Med. Assoc.* 290, 2976–2984.
- Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N.C., Parisi, A., Greco, G., Bellacicco, A.L., Virgilio, S., Celano, G.V., 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int. J. Food Microbiol.* 117, 219–222.
- O'Brien, A.M., Hanson, B.M., Farina, S.A., Wu, J.W., Simmering, J.E., Wardyn, S.E., Forshey, B.M., Kulick, M.E., Wallinga, D.B., Smith, T.C., 2012. MRSA in conventional and alternative retail pork products. *PLoS One* 7 (1), e30092.
- Overesch, G., Büttner, S., Rossano, A., Perreten, V., 2011. The increase of methicillin-resistant *Staphylococcus aureus* (MRSA) and the presence of an unusual sequence type ST49 in slaughtered pigs in Switzerland. *BMC Vet. Res.* 7, 30.
- Pan, A., Battisti, A., Zoncada, A., Bernieri, F., Boldini, M., Franco, A., Giorgi, M., Iurescia, M., Lorenzotti, S., Martinetti, M., Monaci, M., Pantosti, A., 2009. Community-acquired methicillin-resistant *Staphylococcus aureus* ST398 infection, Italy. *Emerg. Infect. Dis.* 15 (5), 845–847.
- Petinaki, E., Spiropoulou, L., 2012. Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. *Clin. Microbiol. Infect. Dis.* 18, 626–634.
- Sergio, D.M.B., Koh, T.H., Hsu, L., Ogden, B.E., Goh, A.L.H., Chow, P.K.H., 2007. Investigation of methicillin-resistance *Staphylococcus aureus* in pigs used for research. *J. Med. Microbiol.* 56, 1107–1109.
- Smith, T.C., Male, M.J., Harper, A.L., Kroeger, J.S., Tinkler, G.P., Moritz, E.D., Capuano, A.W., Herwaldt, L.A., Diekema, D.J., 2008. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in mid-western U.S. swine and swine workers. *PLoS One* 4, e4258.
- Soavi, L., Stellini, R., Signorini, L., Antonini, B., Pedroni, P., Zanetti, L., Milanesi, B., Pantosti, A., Matterelli, A., Pan, A., Carosi, G., 2010. Methicillin-resistant *Staphylococcus aureus* ST398, Italy. *Emerg. Infect. Dis.* 16 (2), 346–348.
- Stegger, M., Liu, C.M., Larsen, J., Soldanova, K., Aziz, M., Contente-Cuomo, T., Petersen, A., Vandendriessche, S., Jimenez, J.N., Mammina, C., van Belkum, A., Salmenlinna, S., Laurent, F., Skov, R.L., Larsen, A.R., Andersen, P.S., Price, L.B., 2013. Rapid differentiation between livestock-associated and livestock-independent *Staphylococcus aureus* CC398 clades. *PLoS One* 14:8 (11), e79645.
- Strommenger, B., Kettlitz, C., Weniger, T., Harmsen, D., Friedrich, A.W., Witte, W., 2006. Assignment of *Staphylococcus* isolates to groups by spa typing, Smal macrorestriction analysis, and multilocus sequence typing. *J. Clin. Microbiol.* 44 (7), 2533–2540.
- Sunde, M., Tharaldsen, H., Marstein, L., Haugum, M., Norström, M., Jakobsen, T., Liim, B., 2011. Detection of methicillin-resistant *Staphylococcus aureus* sequence type 8 in pigs, production environment, and human beings. *J. Vet. Diagn. Investig.* 23, 248–250.
- van Cleef, B.A.G.L., Broens, E.M., Voss, A., Huijsdens, X.W., Züchner, L., Van Benthem, B.H.B., Kluytmans, J.A.J.W., Mulders, M.N., Van De Giessen, A.W., 2010. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. *Epidemiol. Infect.* 138, 756–763.
- van Cleef, B.A.G.L., van Benthem, B.H.B., Verkade, E.J.M., van Rijen, M., Kluytmans-van den Bergh, M.F.Q., Schouls, L.M., Duim, B., Wagenaar, J.A., Graveland, H., Bos, M.E.H., Heederik, D., Kluytmans, J.A.J.W., 2014. Dynamics of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* carriage in pig farmers: a prospective cohort study. *Clin. Microbiol. Infect.* <http://dx.doi.org/10.1111/1469-0919.12582>.
- van Loo, I.H.M., Diederend, B.M.W., Savelkoul, P.H.M., Woudenberg, J.H.C., Roosendaal, R., van Belkum, A., Lemmans-den Toom, N., Verhulst, C., van Keulen, P.H.J., Kluytmans, J.A.J.W., 2007. Methicillin-resistant *Staphylococcus aureus* in meat products, The Netherlands. *Emerg. Infect. Dis.* 13 (11), 1753–1755.
- Vanderhaeghen, W., Hermans, K., Haesebrouck, F., Butaye, P., 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food producing animals. *Epidemiol. Infect.* 138, 606–625.
- Virgin, J.E., Van Slyke, T.M., Lombard, J.E., Zadoks, R.N., 2009. Methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. *J. Dairy Sci.* 92, 4988–4991.
- Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin resistant *Staphylococcus aureus* in pig farming. *Emerg. Infect. Dis.* 11, 1965–1966.
- Wendlandt, S., Schwarz, S., Silley, P., 2013. Methicillin resistant *Staphylococcus aureus*: a food-borne pathogens? *Annu. Rev. Food Sci. Technol.* 4, 117–139.
- Werthlein, H.F., Vos, M.C., Boelens, H.A., Voss, A., Vandenbroucke-Grauls, C.M., Meester, M.H., Kluytmans, J.A., van Keulen, P.H., Verbrugh, H.A., 2004. Low prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) at hospital admission in The Netherlands: the value of search and destroy and restrictive antibiotic use. *J. Hosp. Infect.* 56, 321–325.
- Witte, W., Strommenger, B., Stanek, C., Cuny, C., 2007. Methicillin resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. *Emerg. Infect. Dis.* 13, 255–258.
- Wulf, M., van Nes, A., Eikeleboom-Boskamp, A., de Vries, J., Melchers, W., Klaassen, C., Voss, A., 2006. Methicillin resistant *Staphylococcus aureus* in veterinary doctors and students, The Netherlands. *Emerg. Infect. Dis.* 12, 1939–1941.
- Zhang, S., McClure, J.A., Elsayed, S., Louse, T., Conly, J.M., 2005. Novel multiplex-pcr assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* 43, 5026–5033.