Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates in food industry workers


**Key words:** Methicillin-resistant *Staphylococcus aureus*, food industry workers, food safety

**Parole chiave:** *Staphylococcus aureus* meticillino-resistente, operatore settore alimentare, sicurezza alimentare

Abstract

**Background.** Methicillin-resistant *S. aureus* (MRSA) is a pathogen spread not only in the hospital environment but also in the community and amongst livestock (LA-MRSA). LA-MRSA can be transmitted to humans that live in close contact with MRSA-colonized animals, and human colonization and/or infection has been reported worldwide, particularly among those involved with livestock farming. In this study the authors evaluated the prevalence of *S. aureus* and MRSA among healthy carriers who worked in the food industry in Apulia, Southern Italy.

**Methods.** Nasal swabs were taken from pasta and pork industry workers. All swab samples were subjected to tests for the isolation, identification and typing of *S. aureus* and MRSA strains. The identification of the strains was confirmed by molecular assessment using multiplex-PCR for the amplification of the nuc and mecA genes. The strains identified as MRSA were then subjected to a PCR protocol for the characterization of sequence type ST398.

**Results.** In total 26.3% of examined nasal swabs were positive for *S. aureus*, 8.2% of them were methicillin resistant strains and 28.5% of MRSA isolates were characterized as ST398. The MRSA prevalence among pork factory workers was 3% , whereas among the pasta operators the prevalence was 11.5%.

**Conclusions.** The presence of *S. aureus* and MRSA among food workers represents a public health risk. Further, considering the dissemination of *S. aureus* and MRSA among non-nosocomial environments, including communities and livestock, careful surveillance and continuous monitoring of the emergence of MRSA is fundamental for safeguarding public health.
**Introduction**

*Staphylococcus aureus* is a commensal and opportunistic pathogen of humans and many homoeothermic species (1). Although *S. aureus* colonizes around 32% of individuals, with the anterior nares being the most common site for colonization (2, 3), this microorganism can cause serious and life-threatening infections. Furthermore, some *S. aureus* strains can produce enterotoxins that, when consumed in contaminated food, can be responsible for foodborne outbreaks. In fact, *S. aureus* foodborne illness is one of the most frequently reported diseases worldwide (4, 5).

It has been estimated that over 40% of cases of foodborne illness are caused by human handling of food (6, 7). Infection/colonization of livestock or farm workers have also been described as sources of contamination of food with *S. aureus*.

During the last 20 years, methicillin-resistant *S. aureus* (MRSA) strains have emerged in hospitals and care facilities and have become a serious public health problem. Currently, MRSA is a pathogen spread not only in the hospital environment (termed hospital-associated MRSA), but also in the community (community-associated MRSA) and amongst livestock (livestock-associated MRSA or LA-MRSA). A variety of animals have been found to serve as reservoirs for LA-MRSA, including horses, cattle, dogs, cats (4, 8) and especially pigs (9).

LA-MRSA can be transmitted to humans that live in close contact with MRSA-colonized animals (10), and human colonization and/or infection has been reported worldwide, particularly among those involved with livestock farming (11, 12).

In Europe and America, the majority of LA-MRSA strains belong to sequence type (ST) 398, while in other regions such as Asia, ST9 is more frequently isolated (13, 14). ST398 is normally found in pig and cattle farms (15), but can also be found in poultry, where other sequence types such as ST5 and ST9 are similarly common (16–18).

The aim of this study was to evaluate the prevalence of *S. aureus* and MRSA among healthy carriers who work in the food industry in Apulia in southern Italy, and to analyze the prevalence of methicillin resistance among these isolates.

**Materials and methods**

**Sampling**

From 2013 to 2014, nasal swabs were taken from 323 pasta and pork industry workers. Prior to enrollment in the study, verbal screening was conducted to determine eligibility. Workers were invited to participate in the study if they had been employed by the farm for at least 6 months, they had no evidence of respiratory infection, and had not received antimicrobial therapy in the last 30 days. All eligible participants after clarification of the purposes of the study signed the informed consent approved by the Ethics Committee with its territorial authorization to process data in privacy. Samples from the participants’ external nares were collected using sterile cotton swabs. All swab samples were kept cool in an ice box, transported to the laboratory and immediately subjected to tests for the isolation, identification and typing of *S. aureus* and MRSA strains.

**Isolation of S. aureus and MRSA**

All swabs were streaked onto selective and differential Mannitol Salt agar (MSA; Biolife, Milan, Italy) and chromogenic MRSA agar (MRSA; Biomerieux, France), to isolate *S. aureus* and MRSA, respectively. After incubation at 37°C aerobically for 24-48 h, colonies were subjected to a coagulase test. A methicillin-sensitive *S. aureus* strain (ATCC 29213) was included as a control at each phase of the investigation.
Molecular typing of S. aureus and MRSA

Amplification of the nuc and mecA genes

The identification of the strains was confirmed by molecular assessment using multiplex-PCR for the amplification of the nuc and mecA genes. Amplification of the nuc gene identified strains as S. aureus, and amplification of the mecA gene indicated methicillin resistance.

Strains of interest were cultured overnight, then bacterial DNA was extracted from 1 ml of culture broth using the Genomic DNA Isolation kit (RTA Laboratories Ltd., Turkey) according to the manufacturer’s instructions. The extracts were subjected to duplex-PCR using two sets of primers. The first pair of primers amplified the nuc gene: nuc1 (5’-GGGATGATGTTGATACGGTT-3’) and nuc2 (5’-AGCCAAGCCTTGACGACTAAC-3’), producing a fragment of 280 bp; while, the second pair amplified the mecA gene: mec1 (5’-AAAAATCGATGGTTAAAGTGGCGACTACCGGATTTGC-3’) and mec2 (5’-AGTTCTGGCACTACCGGATTTGC-3’), producing a fragment of 533 bp (19). DNA extracted from a methicillin-susceptible S. aureus strain (ATCC 29213) and a MRSA strain (ATCC 33591) were included in the reactions as controls. The reaction mixture (50 μl total) for the duplex-PCR included: 1 μl of DNA extract, 25 μl of Taq Go Green Master Mix (Promega, Italy) and 0.5 μl (25 μM) of each primer. After pre-incubation at 94°C for 10 min, the following amplification protocol of 35 cycles was used: 96°C for 30 s; 60°C for 30 s; 72°C for 90 s and finally 3 min at 72°C. The products of the PCR reaction were visualized on a 1.5% agarose gel using a UV reader (Euroclone TCX-20-M, EEC) (20).

Characterization of S. aureus ST398

The strains identified as MRSA were then subjected to a PCR protocol for the characterization of sequence type ST398. Primers A04R (5’-TCATTTGCTTGGCGTGTAGGT-3’) and A04F (5’-TATCAA CAGCGGCTGACAAAC-3’) were used to amplify a fragment of 317 bp. The amplification protocol included the following steps: 1) denaturation at 95°C for 3 min, 2) amplification by 25 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, and 3) a final extension step at 72°C for 10 min. The products of the PCR reaction were visualized on a 1.5% agarose gel using a UV reader (Euroclone TCX-20-M, EEC) (20).

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software 10 for Mac OS X (SPSS Inc., Chicago, IL, USA). The analysis was based on Fisher’s exact test (two-tailed). The level of significance was set a \( p < 0.05 \).

Results

In total, of 323 nasal swabs examined in this study (162 from pasta operators and 161 from pork factory workers), 85 (85/323; 26.3%) were positive for S. aureus on culturing. Of these isolates, 52 (52/85; 61.2%) were from the pasta operators and 33 (33/85; 38.8%) were from the pork industry workers (Table 1). Based on growth on chromogenic medium, seven of 85 S. aureus strains (7/85; 8.2%) were methicillin resistant.

Of seven operators colonized with MRSA, including five males and two females, none were suffering from respiratory and/or skin infections and none had been treated with antibiotics within 30 days prior to collection. Six of the seven MRSA strains isolated (6/7; 85.7%) were from the pasta industry personnel and only one strain (1/7; 14.3%) was isolated from a pork factory worker.

The MRSA prevalence among pork factory workers was 3% (1/33), whereas among the pasta operators the prevalence was 11.5% (6/52) (Table 1).
Molecular investigations

All *S. aureus* strains were identified by molecular analyses as being positive for the *nuc* gene (Figure 1) and a statistically significant difference was observed between *nuc*+ carriers operating in the pasta industry (52/161; 32%) versus those working in the meat industry (33/161; 20.5%) (*p*= 0.016). Of 85 *S. aureus* isolates, seven (7/85; 8.2%) were found to carry the *mecA* gene and were considered MRSA. A not statistically significant difference was observed between the *mecA*+ carriers operating in the pasta and pork industries (6/52; 11.5% vs. 1/33; 3%) (*p* = 0.239).

Of the seven strains carrying the *mecA* gene, two were characterized as MRSA ST398 (2/7; 28.5%) (Figure 2). Both of these strains were isolated from pasta industry workers.

**Discussion and conclusions**

During the last few decades, the epidemiology of MRSA has changed: strains historically associated with nosocomial infections have also been isolated in community and livestock environments, posing different public health problems. MRSA strains are found to contaminate various types of food including retail pork and beef, and bovine milk and cheese (21–23). It is well documented that the most common causes of contamination are human handling of food products and

<table>
<thead>
<tr>
<th>Samples Nasal Swabs (No)</th>
<th>S. aureus/ nuc+ No (%)</th>
<th>MRSA/ mecA No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasta industry workers (162)</td>
<td>52 (32.1)</td>
<td>6 (11.5)</td>
</tr>
<tr>
<td>Pork industry workers (161)</td>
<td>33 (20.5)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Total (323)</td>
<td>85 (26.3)</td>
<td>7 (8.2)</td>
</tr>
</tbody>
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Table 1 - *S. aureus* and MRSA isolates from nasal swabs that were taken from 323 pasta and pork industry workers.
infection/colonization of livestock or farm workers (21, 24, 25). In the present study, we evaluated the prevalence of *S. aureus* and MRSA in healthy carriers who worked in the food industry either handling pork or pasta. *S. aureus* was isolated from over 26% of operators, with a higher prevalence observed among the pasta sector workers (61% vs. 39%). Of the isolates, 8.2% (7/85) were MRSA strains and 85% of these were isolated from the pasta sector workers. The MRSA prevalence among the pork factory workers was 3% (1/33), whereas among the pasta operators the prevalence was 11.5% (6/52). These results were in agreement with other European studies where MRSA prevalence among at-risk populations, including slaughterhouse workers and veterinarians, was found to range between 3% and 12.5% (26, 27). It is interesting to note that in our study, MRSA strains were more common among pasta operators that had no professional contact with meat. To date, the results in the literature are highly variable in fact they indicate a prevalence of MRSA carrier state from 0 to 38%, mainly concerning the meat sector (5, 28), while there are few data on the distribution of these strains in the other food sectors. However, these results confirm that MRSA strains, spread in livestock operations and hospitals where antibiotics are regularly used, can spread into communities and the environment colonizing persons who are not occupationally exposed (29). Another interesting finding from our study was that two of seven MRSA strains belonged to the ST398 clone and these were isolated from the pasta industry operators. MRSA ST398 is usually isolated from pork, and has also been identified in some domestic animals (including dogs, cattle, and poultry), and in populations with no obvious livestock contact (29).

It is therefore considered a health risk for farmers and those who work closely with animals, but also among persons who do not have direct contact with livestock but reside in areas of high livestock density (30). So, the spread of MRSA ST398 strains between food operators may result from domestic or non-occupational activities. The presence of this bacterium among food workers represents a public health risk and workers may therefore need to be informed of necessary hygiene and control measures. Further research is also needed to identify the causes of the different prevalence levels of MRSA in the community and in particular clone ST398. Based on these results, a deeper understanding of the prevalence and means of transmission of MRSA in the food chain may be gained that could provide useful information in preventing the spread of this organism, allowing for a proper risk assessment to be made in the chain of food production to effectively safeguard the health of producers and consumers.

In conclusion, considering the dissemination of *S. aureus* and MRSA among non-nosocomial environments, including communities and livestock, careful surveillance and continuous monitoring of the emergence of MRSA is fundamental for safeguarding public health.

Riassunto

Prevalenza e caratterizzazione di ceppi di *Staphylococcus aureus* meticillino-resistenti in operatori del settore alimentare

Introduzione. *S. aureus* meticillino resistente (MRSA) è un patogeno diffuso non solo in ambito ospedaliero ma anche comunitario e negli allevamenti (LA-MRSA). Attualmente è ben documentato in tutto il mondo come LA-MRSA possa essere trasmesso all’uomo che si trova in stretto contatto con animali colonizzati. In questo studio gli autori valutarono la prevalenza di *S. aureus* e MRSA tra soggetti portatori sani che lavoravano in due tipologie di industrie alimentari tipiche della Regione Puglia.

Metodi. I tamponi nasali furono eseguiti in lavoratori addetti alla produzione di pasta e di carne suina. Tutti i campioni furono sottoposti ad indagini microbiologiche per l’isolamento, identificazione e tipizzazione dei ceppi
S. aureus e MRSA. L’identificazione dei ceppi fu confermata da indagini molecolari. Gli stipiti riconosciuti come MRSA furono sottoposti a ulteriori indagini molecolari per la caratterizzazione dei ceppi ST398.

Risultati. Il 26,3% dei tamponi analizzati risultarono positivi per S. aureus, 8,2% di essi risultò resistente alla meticillina e il 28,5% degli isolati MRSA furono caratterizzati come ST398. La prevalenza di MRSA risultò 11,5% e 3% rispettivamente nei lavoratori addetti alla produzione di pasta e di carne suina.

Conclusioni. La diffusione di S. aureus e MRSA tra i lavoratori del settore alimentare rappresenta un serio problema di sanità pubblica. Considerando la ampia sorveglianza e un continuo monitoraggio dell’emergenza di questi ceppi risulta fondamentale per tutelare la salute della popolazione.

References


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