Articolo originale

Original article

Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among subjects working on bovine dairy farms

Prevalenza e caratteristiche molecolari di Staphylococcus aureus meticillino-resistente (MRSA) nel personale addetto alla mungitura delle vacche da latte

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INTRODUCTION

Meticillin-resistant Staphylococcus *aureus* (MRSA) is a major cause of healthcare-associated infections worldwide [1]. In addition, MRSA has recently been identified as an emerging pathogen in livestock and companion animals [2-5]. It is a common cause of mastitis in dairy cows and it has been isolated from bulk tank milk [6-8]. Livestock associated MRSA (LA-MRSA), belonging to the clonal complex 398 (CC398), have been found in farmers and food producing animals, especially pigs and calves [9-17]. Several reports have shown parallel occurrence of MRSA CC398 in both animals and humans [3, 12, 14-17], but evidence for animal-to-human transmission of MRSA is often indirect and based on parallel observations in genetic or resistance patterns [18, 19]. Since MRSA CC398 may cause severe infections in humans, contact with people working on livestock might represent a risk factor for the development of MRSA-associated illness [20, 21].
The aim of our study was to evaluate the prevalence of MRSA among dairy farmers of the province of Ragusa (South-Eastern Sicily), their animals and bulk tank milk samples. We characterized MRSA isolates with respect to antimicrobial resistance, virulence-associated genes and ability to form biofilm.

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MATERIALS AND METHODS

Farm sampling

From October to December 2010, we randomly selected forty-five dairy farms in the province of Ragusa (South-Eastern Sicily) among those having experienced MRSA isolation from bulk tank milk in the previous three years. We collected nasal swabs from individuals working or living on farms and from dairy cows; in addition, a bulk tank milk sample was collected on each farm. Participants were asked to fill in a questionnaire containing 15 items about farm hygiene practices associated with milking. Collected samples were transported in coolers to the laboratory (Section of Microbiology, Department of Bio-Medical Sciences, University of Catania, Italy).

Laboratory analysis

Nasal swabs, as well as bulk tank milk samples, were firstly incubated in Brain Heart Infusion (BHI) broth (Oxoid, Italy) with 2% NaCl. After overnight incubation at 37°C, 100 µl of the culture broth were transferred into a selective

Mannitol-Salt-Agar, supplemented with 75 mg/L aztreonam and 6 mg/L oxacilline. All suspected colonies were identified as S. *aureus* using standard techniques: colony morphology, catalase and coagulase test, biochemical tests (Api-Staph system, bioMérieux, Italy). Strains with ambiguous results in phenotypic tests were analyzed by 16S rRNA gene sequencing [22]. Methicillin resistance was evaluated by the cefoxitin disk diffusion method and correlated with the presence of mecA gene [23]. DNA was extracted using a QIAamp DNA minikit (Qiagen, MD) per manufacturer's instructions. Molecular characterization of all strains was conducted by polymerase chain reaction (PCR) of mecA, Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin (TSST) genes. Accessory gene regulator (agr) specificity grouping was carried out for all isolates. *S. aureus* isolates were screened for their antimicrobial susceptibility patterns using the disk diffusion method. The tested antibiotics were rifampin (5 µg in disk), cefoxitin (30 µg), erythromycin (15 µg), mupirocin (5 µg), tetracycline (30 µg), gentamicin (10 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole (25 µg) and ciprofloxacin (5 µg) (Oxoid, Italy). Interpretations of results as susceptible or resistant were done according to the Clinical and Laboratory Standards Institute guidelines [24]. Biofilm production was determined as previously published [25]. S. *aureus* isolates were considered strong biofilm producers if they had A490>0.4, medium biofilm producers if they had A490 ranging from 0.2 to 0.4, weak biofilm producers if they had A490 <0.2. *S. epidermidis* ATCC35984 served as a positive control.

Statistics

Data were entered into an Excel database. Categorical variables were analyzed by using Fisher's exact test. Intergroup comparisons were performed using Mann-Whitney U test.

RESULTS

A total of 622 samples were collected and tested for the presence of MRSA. Nasal swabs were taken from 113 individuals and 461 dairy cows (10-15 livestock units for each farm, on the basis of farm size); in addition, 48 samples were taken from bulk tank milk. MRSA prevalence rate was 55% (344/622 samples); 61% (283/461) of bovine samples tested positive for MRSA, in comparison with 36% (40/113) of humans and 44% (21/48) of bulk tank milk samples (Figure 1). MRSA prevalence was significantly higher among men in comparison with women (39.8% vs. 6.6%, p<0.05). No association was found with age, nationality and duration of animal contact. MRSA carriage in humans was associated with the prevalence of MRSA in dairy cattle and milk samples. In fact, MRSA carrier prevalence in humans significantly correlated with the percentage of positive cows on the farm (p<0.01) and with the number of livestock units (p<0.05). In addition, it was more likely to find an increased prevalence of MRSA carriers among humans on farms with milk positive for MRSA (62.5% vs. 32.9%, p<0.01). A negative correlation was found between somatic cell count (SCC) and milking hygiene score (r=-0.45). The characteristics of study participants and farms are described in Table 1.

Only one farm tested totally negative for MRSA (cattle, humans and milk samples). In 14 farms,

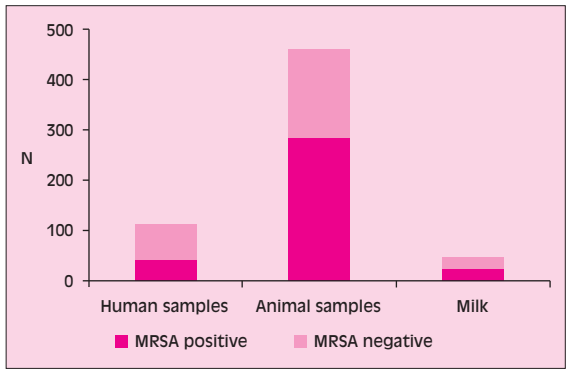

Figure 1 - MRSA prevalence rate among farmers, cattle and bulk tank milk samples. 61% of bovine samples tested positive for MRSA, in comparison with 36% of humans and 44% of bulk tank milk samples.

Table 1 - Characteristics of the study participants and farms.

Age (years)**	40 (27-53)
Gender (Male/Female)*	98 (86.7)/15 (13.3)
Nationality (Italian/Not Italian)*	93 (82.3)/20(17.7)
Animal exposure (years)**	15 (4-33)
Livestock units**	50 (35-80)
Milking hygiene score**	14 (10-17)
Somatic cell count (cells/µl)**	350 (223-500)

*N (%) **Median (Interquartile range).

nasal swabs from dairy cows were positive for MRSA, 5 farms tested positive for both animals and milk samples, in 10 farms nasal swabs were positive among humans and animals. Lastly, 15 farms tested positive for humans, cattle and milk samples. Human and milk MRSA isolates from these 15 farms were screened for the agr locus, TSST gene and the PVL toxin gene; in addition, we evaluated their capability to produce biofilm. 71.5% of milk isolates were associated with the agr locus type I and were medium biofilm producers; 28.5% were weak biofilm producers belonging to the agr locus type III. As for human isolates, 28.5% (agr locus type II and type III) were medium biofilm producers, 71.5% (57.1% belonging to agr locus type I and 14.4% to agr locus type II) were weak biofilm producers. All isolates were negative for the gene encoding PVL; no human MRSA was associated with the presence of TSST gene, whereas two milk isolates (agr locus I) harboured the gene encoding for TSST. All human and milk isolates were susceptible to erythromycin and mupirocin and resistant to sulfamethoxazole/trimethoprim. 28.5% of both human and milk isolates were resistant to rifampin. Resistance to tetracycline or clindamycin was found in 42.8% of human MRSA vs. 28.5% of milk MRSA. All milk isolates were resistant to ciprofloxacin, in comparison with 71.4% of human MRSA. 42.8% of milk MRSA was susceptible to gentamicin, compared to 28.5% of human isolates.

DISCUSSION

In the present study, we found a very high rate of MRSA nasal colonization among people occupationally in contact with bovine dairy cattle. In fact, 36% of nasal swabs taken from farmers tested positive for MRSA, which is significantly higher than the general population. Other reports have shown a lower prevalence of MRSA carriers among farmers. Graveland et al., for instance, found that 15.9% of persons living and working on veal calf farms were positive for MRSA [13].

As refers to MRSA isolation from bulk tank milk, our results are in contrast with available literature, because we found a significantly higher rate of positive isolates (44%) in comparison with previous studies [7, 26, 27]. Spohr et al. found that milk samples of 5.1-16.7% of dairy cows were positive for MRSA; Virgin et al. did not identify MRSA from bulk tank milk [7, 26]. Our observation may be partially due to our choice to select farms among those having experienced MRSA isolation from bulk tank milk in the previous three years. Considering that the milking hygiene score correlated with the somatic cell count, which is increased in the presence of mastitis, it is supposable that the improvement of hygiene practices might reduce the risk for MRSA to spread on dairy farms, for example via milkers hands and milking clusters, which represent a common route of transmission for mastitis pathogens, especially S. *aureus* between cows.

It is remarkable to note that in our study MRSA carriage among humans paralleled MRSA isolation from their cattle, thus in keeping with the aforementioned paper of Graveland et al. and consistent with the possibility of transmission between animals and people who are in close contact with them [13]. A high rate of animal-to-human transmission of CC398 has been reported in pig farming, as well as a significant difference in MRSA prevalence between farmers and their families [14-17]. Koch et al. found that contact with pigs was associated with the risk for MRSA CC398 colonization in a retrospective study among patients admitted to a tertiary-care university hospital [28]. In addition to classical risk factors for MRSA carriage, the authors suggested to include the evaluation of contact with livestock as an additional risk factor to the admission screening schedule for hospitals, in order to identify subjects at higher risk for LA-MRSA colonization, who may be responsible for MRSA CC398 introduction in the nosocomial setting and may favor antimicrobial resistance import to hospitals [29].

In conclusion, our study showed a very high prevalence of MRSA colonization among dairy farmers and cows in the South-Eastern Sicily. Considering that MRSA can represent a public health concern, we believe that prospective studies are needed in order to better explore MRSA transmission between animals and humans; in addition, it would be worthy to implement preventive strategies and to monitor the resistance profile of S. *aureus* within and beyond the farm environment.

Keywords: MRSA, milk, *Staphylococcus aureus*, livestock, dairy cattle.

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare-associated infections worldwide and has recently been identified as an emerging pathogen in livestock and companion animals.
Livestock-associated MRSA (LA-MRSA) may be responsible for increased rates of colonization and/or infection among people working on farms. We evaluated the prevalence and molecular characteristics of MRSA among dairy farmers in the province of Ragusa, South-Eastern Sicily, their animals and bulk tank milk samples. A surprisingly high number of samples tested positive for MRSA: 36% of human nasal swabs, 61% of bovine nasal swabs and 44% of bulk tank milk samples. MRSA carrier prevalence in humans significantly correlated with the percentage of positive cows on the farm, the number of livestock units and the presence of consensual positive bulk tank milk samples. Prospective studies are needed to investigate MRSA transmission between animals and humans and implement preventive strategies.

RIASSUNTO

Lo Staphylococcus aureus *meticillino-resistente (MRSA) rappresenta la causa principale di infezioni nosocomiali a livello mondiale.*
Il CC398 è il ceppo di MRSA più frequentemente associato allo stato di portatore asintomatico negli animali destinati alla produzione alimentare in allevamenti intensivi (LA-MRSA). Le persone a contatto con il bestiame sono esposte ad un rischio maggiore di colonizzazione e infezione da MRSA rispetto alla popolazione generale.
L'obiettivo del nostro studio è stata la valutazione della prevalenza e del profilo molecolare di MRSA nel personale addetto alla mungitura delle vacche da latte nella provincia di Ragusa (Sicilia sud-orientale), negli animali da loro allevati e nelle vasche di raccolta del latte.
Un numero sorprendentemente elevato di campioni sono risultati positivi per MRSA: 36% dei campioni nasali umani, 61% dei campioni nasali bovini e il 44% dei campioni prelevati dalle vasche del latte. Lo status di portatore tra gli addetti alla mungitura correlava con la percentuale di animali positivi nell'allevamento, con il numero di capi di bestiame e con il riscontro di positività nelle vasche del latte.
Studi prospettici sono necessari per valutare meglio la trasmissione di MRSA tra animale e uomo e per implementare adeguate strategie di prevenzione.

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