Short Communications

Long-term study of MRSA ST1, t127 mastitis in a dairy cow

R. Pilla, V. Castiglioni, M. E. Gelain, E. Scanziani, V. Lorenzi, M. Anjum, R. Piccinini

METICILLIN-resistant *Staphylococcus aureus* (MRSA) has been reported in human medicine as a cause of nosocomial and community-associated infections (Otter and French 2010). In veterinary medicine, MRSA strains have been identified in a wide range of animals and diseases (Leonard and Markey 2005, Fessler and others 2009, Huber and others 2010, Türkylmaz and others 2010), thus it is considered an emerging threat with a high zoonotic potential (Juhasz-Kaszanyitzky and others 2007). MRSA sequence type (ST) 1, spa type (t) 127 has been mostly isolated from community-associated infections, but t127 has seldom been identified in cattle and pigs (Haslam and others 2010).

The present report regards a dairy cow with an intramammary infection by MRSA ST1, t127 that was investigated over an entire lactation and submitted to histological evaluation of mammary tissue to explore both bacterial molecular features and host immune response in the milk and mammary tissue. The study was performed because only a few mammary infections of the dairy cow by this particular strain have been reported and no information is available on the interaction between the bacteria and the mammary gland.

A five-year-old Holstein-Friesian dairy cow from a closed herd located in Milano province, Italy, consisting of 25 lactating cows, showed an intramammary infection with *S. aureus* in both right quarters. The isolate revealed a multidrug resistance profile by the agar plate method, and PCR analysis (Costa and Palladini 2005) confirmed the presence of mecA genes in both isolates, which were consequently classified as MRSA. The farmer decided to antibiotic treat and dry off the animal, despite the authors’ suggestion to cull it. The therapy consisted of both a first and a fourth-generation cephalosporin.

After parturition, the animal was sampled three times during the lactation, scheduled as follows: one week after parturition, in the middle of lactation and three weeks before culling. Each sampling period lasted for five days, and aseptic milk samples were collected daily from each mammary quarter.

In the final sampling, cytometric analysis of milk cells from the four mammary quarters was also performed, as described by Koess and Hamann (2006), using an antibody panel of bovine surface antigen (Table 1). Milk cells were acquired on a flow cytometer (FACScalibur, Becton Dickinson) and analysed using Cell Quest software (Becton Dickinson).

At the slaughterhouse, parenchymal tissue was collected from each mammary quarter for bacteriological analysis. Samples were also fixed in 10 per cent buffered formalin, progressively dehydrated and embedded in paraffin, for histological and immunohistochemical evaluation. Immunohistochemical staining was performed using a panel of antibodies as indicated in Table 1, and the results were quantified by image analysis, with Image Pro Plus Software Technology (Media Cybernetics).

Bacteriological analysis was performed as described elsewhere (Hogan and others 1999), and presumptive identification of bacterial isolates was confirmed by the API System (bioMérieux). Somatic cells were counted on a Bentley Somacount 150 (Bentley Instruments).

The infection was confirmed only in the right forequarter, and the shedding of the microorganism was irregular: it could be detected in all milk samples from the first and third follow-up periods, but only on day 1 of the second period. Bacterial counts ranged between 25 and 2700 cfu/ml milk. Throughout the study, the somatic cell count (SCC) in the MRSA-infected quarter fluctuated between 300 and 6000 cells/μl, which was not in parallel with the shedding of MRSA. After culling, MRSA was detected in the mammary tissue of the infected quarter.

The results of cytometric analysis are shown in Fig 1. In both right quarters, PMNs were always high (60 to 75 per cent), except for the front right quarter on the second sampling, while the left quarters demonstrated high lymphocyte and low phagocytes levels. T cells were always the predominant lymphocyte subset present, with a CD4/CD8 ratio above 1.

Histological examination showed a moderate amount of lymphocytes and plasma cells in the stroma of all quarters. Multifocal areas characterised by the presence of groups of neutrophils were evidenced in alveolar lumens of the infected quarter (Fig 2a). Image analysis confirmed that the right hindquarter had the highest scores for myeloperoxidase (Fig 2b). The area of immunolabelled cells was 16,035 μm², followed by 4319 μm² in the ipsilateral quarter; the values in the left quarters were 1945 and 1963 μm².

All the MRSA isolates were genotyped at the Istituto Superiore di Sanità by MLST and spa typing: they belonged to ST1 complex and were spa t127. All isolates were further genotyped by DNA microarray (CLONDIAG chip technology, Jena), as described elsewhere (Monecke and Ehrlich 2005). Overall presence of β-lactamase genes

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**TABLE 1: Antibody panel used to differentiate milk cells (above the line space); FITC fluorescein isothiocyanate, PE phycoerythrin. Antibody panel used for immunohistochemical staining (below the line space)**

<table>
<thead>
<tr>
<th>CD molecule</th>
<th>Antibody type</th>
<th>Specificity</th>
<th>Antibody clone</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b-FITC</td>
<td>Mouse IgG2b</td>
<td>Granulocytes</td>
<td>CC126</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>CD14-PE</td>
<td>Mouse IgG2a</td>
<td>Monocytes</td>
<td>TUK4</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>CD21-PE</td>
<td>Mouse IgG1</td>
<td>B lymphocytes</td>
<td>CC21</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>CD4-FITC</td>
<td>Mouse IgG1</td>
<td>T lymphocytes</td>
<td>CC17</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>CD3-PE</td>
<td>Mouse IgG2a</td>
<td>T helper lymphocytes</td>
<td>CC8</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>CD4-PE</td>
<td>Mouse IgG2a</td>
<td>T cytotoxic lymphocytes</td>
<td>CC63</td>
<td>Ab Serotec, UK</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>Rabbit polyclonal</td>
<td>Granulocytes</td>
<td>N1578</td>
<td>Dako, DK</td>
</tr>
<tr>
<td>CD79alpha</td>
<td>Mouse monoclonal</td>
<td>B lymphocytes</td>
<td>M7051</td>
<td>Dako, DK</td>
</tr>
<tr>
<td>CD3</td>
<td>Rabbit polyclonal</td>
<td>T lymphocytes</td>
<td>A0452</td>
<td>Dako, DK</td>
</tr>
</tbody>
</table>

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and of the genes for resistance to macrolides and aminoglycosides was revealed. All the isolates also carried genes for α-haemolysins and β-haemolysins, leukocidin D/E ( luk D/E), both components of γ-haemolysin, enterotoxin H and the superantigen X.

S. aureus is a major cause of chronic subclinical mastitis in dairy cows (Young and others 2001), and MRSA is considered an emerging pathogen of the mammary gland. The presence of this MRSA type suggests a human-cow transmission as previously reported (Juhász-Kaszanyitzky and others 2007), since the herd was a closed one without recent introduction of new animals, and the cow had never been moved. ST1, t127 has been described as a human community-associated MRSA strain that can be Panton-Valentine leukocidin (PVL)-positive or PVL-negative (Oter and French 2010). Accordingly, the MRSA found in the present study carried several genes coding for different bicomponent toxins, but not for PVL.

Shedding of MRSA from the infected quarter was irregular and the immune response indicated an acute infection, with the major cell population always represented by PMN, even though MRSA infection was chronic. This result was in contrast to what has been observed in S aureus-induced chronic mastitis, in which macrophages were the principal inflammatory cell type detectable in epithelial lining, in lumens and in the glandular interstitium (Foster 2007). In the left quarters, the lymphocytes accounted for 50 to 70 per cent of the cells and T lymphocytes were overall the predominant population, in accordance with other authors (Taylor and others 1997, Ebling and others 2001). The multifocal distribution of inflammatory infiltrates revealed by histological analysis reflected the patchy progression of S aureus mastitis described in current literature (Schafer and Miller 2007) and may suggest that clearance of a single inflammatory focus occurred, while others remained active or developed over time. As a result, SCC and MRSA counts fluctuated irregularly during the follow-up periods, sometimes showing low SCC values despite the chronic course of infection. The actual consequence is that misdiagnosis can occur. Thus, the authors suggest that all cases of recurrent mastitis by S aureus should be screened for meticillin resistance.

Acknowledgement

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References


FIG 1: Milk cell profiles in the third sampling period. (a), (c), (e) and (g) show the relative percentages of lymphocytes (L), polymorphonuclear cells (PMN) and macrophages (M). (b), (d), (f) and (h) show the lymphocyte profile (T cells, B cells, CD4+ T helpers, CD8+ T cytotoxic and natural killers). Roman numerals indicate the days of sampling.

FIG 2: Histological and immunohistochemical analyses of mammary parenchyma from infected quarter. (a) Alveolar lumens filled by large numbers of both viable and degenerated (karyorrhectic) neutrophils and lined by a fully active secretory epithelium. Haematoxylin and eosin, x200. (b) Hot spot field with alveolar lumens filled by neutrophils that are positive for myeloperoxidase (Mpo). Mpo staining. x200.
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