Staphylococcal bovine mastitis in France: enterotoxins, resistance and the human Geraldine methicillin-resistant Staphylococcus aureus clone

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Sir,

Staphylococcus aureus, which potentially produces toxins that are common sources of food poisoning worldwide, represents about one-third of the bacteria isolated from bovine mastitis in France.1 Recently, in this country, a food poisoning outbreak due to the presence of the staphylococcal enterotoxin E in cheese made from unpasteurized cow’s milk was reported.2 This prompted us to retrospectively characterize a collection of S. aureus associated with bovine mastitis in order to detect the presence of enterotoxin genes and resistance phenotypes that might be of public health concern.

Between February 2007 and June 2008, 139 non-duplicate S. aureus isolates from bovine mastitis were collected from 15 French veterinary laboratories covering all main cattle-rearing areas. Isolates were sent to the national reference laboratory of the Anses (French agency for food, environmental and occupational health safety) in Lyon, France, and identified using a triplex PCR targeting the 16S rRNA, mecA and S. aureus-specific nuc genes. Antimicrobial susceptibility was tested by the disc diffusion method on Mueller–Hinton agar and interpreted according to the breakpoints recommended by the Antibiotic Committee of the French Society of Microbiology (http://www.sfm.asso.fr). Sixteen antibiotics of veterinary and/or human interest were tested, using S. aureus ATCC 25923 as the quality control strain. Finally, the genes sea to see and seg to seq (enterotoxins), eta and etb (exfoliative toxins), tst (toxic shock syndrome toxin) and lukS-PV-lukF-PV (Panton–Valentine toxin) were screened by PCR.3

Enterotoxin genes were detected in 68.3% (95/139) of the isolates, and 54.0% (75/139) harboured a combination of two to four genes, with one strain even presenting a combination of six genes (Table 1). The genes sea, see, eta, etb and lukS-PV-lukF-PV were not detected, whereas sea, encoding the most frequent enterotoxin associated with food poisoning, was detected in 5 isolates (3.6%). In contrast, seg and sei were found in 70 and 71 isolates, respectively, and co-occurred in 61 isolates (associated or not with other toxins). Both genes are part of the enterotoxin gene cluster (sea, selm, sei, seln and seg), which has often been associated with S. aureus from bovine mastitis. The combination of sed and sej, usually localized on a plasmid, was also detected in 10 isolates (7.2%). Finally, tst and sec, which are part of the bovine S. aureus pathogenicity island SAPIbov, were detected in 12 isolates (while tst was most probably not associated with SAPIbov in the strain presenting six virulence genes).

The proportion of penicillin-resistant isolates reached 41.0%, but antimicrobial resistance was otherwise weak, with only 10 isolates (7.2%) resistant to tetracycline and 9 to macrolides/lincosamides/streptogramin B (MLSβ phenotype; 6.5%). One isolate was resistant to penicillin, cefoxitin, kanamycin and tetracycline, but susceptible to all other antibiotics, including gentamicin. Since methicillin-resistant S. aureus (MRSA) is very rare in cases of bovine mastitis in France, this isolate was further characterized using a microarray-based assay (S. aureus Genotyping, Identibac–Alere) that simultaneously allows (i) detection of clinically relevant virulence factors, resistance determinants and typing markers of S. aureus and (ii) assignment of isolates to strains, clones or clonal complexes.4 Bovine MRSA isolates have been described only rarely,5–7 but there are recent reports of mastitis associated with MRSA ST398 in Germany, which presented only very few virulence genes as characterized by the same microarray technique,6 and in Belgium.8 Here, surprisingly, the MRSA was assigned to the human epidemic Geraldine clone, which has emerged in France since 2007.10,11 Indeed, sequencing revealed a ST5 strain belonging to the spa-type t002, which perfectly matched all the characteristics of the Geraldine clone, including the accessory gene regulator allele (agr2) and the SCCmec cassette type 1 (ccrA1, ccrB1), as well as the complete patterns of resistance (mecA, blaK, blal, blaZ, aadD, tet(L) and fosB) and virulence (tst, sec, sed, sei, sem, seo, clfA-B, epbS, eno and efb) genes. The immune evasion cluster, which is rarely found in animals and is typical of human adaptation, and which is thought to be involved in the success of this particular clone, was also detected. Thus, the presence of the Geraldine clone in a case of bovine mastitis, isolated in August 2007 in the Orne district, strongly argues for on-farm human transmission to the cow. This is of great concern since S. aureus is known to easily transmit within a farm and classically causes contagious mastitis. Moreover, resistance to methicillin (and multiresistance) makes it so difficult to treat that...
veterinarians tend to use broad-spectrum or last-generation anti-
biotics, thus increasing the risk of resistance.

In conclusion, this study showed that the vast majority of the
isolates from bovine mastitis presented neither a worrying resist-
ance phenotype nor the most common enterotoxins causing
food poisoning outbreaks in humans (i.e. sea, seb and sed), indi-
cating that the risk to health as a result of consuming unpasteur-
ized milk or its derivatives is low. The only isolate that clearly
diverged, in terms of both resistance and virulence, was the
MRSA Geraldine clone, which is the only sporadic case of MRSA
reported in 2007–08 in bovine mastitis in France. Worldwide,
the prevalence of MRSA is increasing in humans, with the occur-
rence of community-acquired MRSA, and in animals, especially
due to the widely distributed ST398 clone. However, even
though the detection of highly related MRSA isolates in cattle
and humans has already been documented, the routes of
transmission of MRSA from human to cow, underlining the risk from cross-contamination with strains that
may be selected at each passage for increased resistance or
virulence.

Acknowledgements
We gratefully thank all the department laboratories of the Resapath
network that participated in this study.

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and occupational health safety (Anses).

Table 1. Combinations of virulence genes and resistance patterns detected in the 139 S. aureus isolates

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>No. of isolates</th>
<th>Percentage</th>
<th>Resistance patterns (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>2</td>
<td>1.4</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>sed</td>
<td>1</td>
<td>0.7</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>seg</td>
<td>7</td>
<td>5.0</td>
<td>PEN, TET (1)</td>
</tr>
<tr>
<td>seh</td>
<td>2</td>
<td>1.4</td>
<td>PEN, KAN, ERY, TET (1)</td>
</tr>
<tr>
<td>sei</td>
<td>8</td>
<td>5.8</td>
<td>PEN (4)</td>
</tr>
<tr>
<td>sea-sed</td>
<td>1</td>
<td>0.7</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>sed-sej</td>
<td>7</td>
<td>5.0</td>
<td>PEN (7)</td>
</tr>
<tr>
<td>seg-sei</td>
<td>46</td>
<td>33.1</td>
<td>PEN (4)/PEN, ERY (1)/PEN, cMLSb (1)/PEN, TET (1)</td>
</tr>
<tr>
<td>seg-sej</td>
<td>1</td>
<td>0.7</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>sea-sed-sej</td>
<td>2</td>
<td>1.4</td>
<td>PEN, ERY (1)</td>
</tr>
<tr>
<td>sec-seg-sei</td>
<td>1</td>
<td>0.7</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>sed-seg-sei</td>
<td>1</td>
<td>0.7</td>
<td>PEN (1)</td>
</tr>
<tr>
<td>seg-tst</td>
<td>1</td>
<td>0.7</td>
<td>—b</td>
</tr>
<tr>
<td>sec-sei-tst</td>
<td>2</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td>seg-sei-tst</td>
<td>2</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td>sec-seg-sei-tst</td>
<td>10</td>
<td>7.2</td>
<td>—</td>
</tr>
<tr>
<td>sec-sed-seg-sei-sej-tst</td>
<td>1</td>
<td>0.7</td>
<td>PEN, FOX, KAN, TOB (1)</td>
</tr>
<tr>
<td>None</td>
<td>44</td>
<td>31.7</td>
<td>PEN (20)/cMLSb (1)/PEN, TET (5)/PEN, ERY (1)/PEN, cMLSb (1)/PEN, TET, cMLSB (2)</td>
</tr>
</tbody>
</table>

acMLSb, constitutive MLSb; ERY, erythromycin; FOX, cefoxitin; KAN, kanamycin; PEN, penicillin; TET, tetracycline; TOB, tobramycin.

b—, no associated resistance.

Transparency declarations
None to declare.

References
pUO-SeVR1 is an emergent virulence–resistance complex plasmid of Salmonella enterica serovar Enteritidis

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Sir, Salmonella enterica serovar Enteritidis (Salmonella Enteritidis) is the most common serovar isolated from humans in Europe, Asia and South America (http://www.who.int/salmsurv/links/GSSPProgressReport2005.pdf). Although the occurrence of antimicrobial drug resistance (R) in this serovar is lower than in others, the percentage of isolates resistant to at least one antimicrobial has increased over the last few years in Europe, and multidrug-resistant (MDR) isolates have also been reported.1 Most Salmonella Enteritidis isolates carry the virulence (V) plasmid pSEV, which has a molecular size of ~60 kb, contains the IncFII and IncFIB incompatibility regions and is not conjugative. In Salmonella Enteritidis, a conjugative plasmid of the IncFIC group, which carried blaTEM1 together with spvCD, has been described.2 In the present work, a new and complex virulence–resistance (VR) plasmid, found in an MDR Salmonella Enteritidis isolate is characterized.

Salmonella Enteritidis CNM4839/03 is a clinical isolate collected in Spain in 2003, which shows phage type 14B and resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline and trimethoprim.3 The strain harbours a class 1 integron with the 700 bp/dfrA7 variable region, and the blaTEM1, catA2, strA–strB, sul1, sul2 and tet(A) genes. CNM4839/03 contains two plasmids, of ~75 and ~100 kb (Figure S1, available as Supplementary data at JAC Online). None shows the size of pSEV but Southern blot hybridizations,4 with probes specific for spvC, blaTEM1, and the variable region of the integron, revealed their location on the ~100 kb plasmid, which was hence identified as a new VR hybrid plasmid and designated pUO-SeVR1.

To further characterize the hybrid plasmid, conventional conjugation experiments using Escherichia coli K12 JS3 as recipient were carried out.3 With this procedure, transconjugants were obtained at very low frequency and only after overnight conjugation in liquid broth. These transconjugants harboured a single plasmid of ~180 kb where spvC and the integron mapped (not shown), and could have resulted from co-integration between the ~75 and ~100 kb plasmids. However, by triparental mating5 the conjugative pRK2013 plasmid carried by the helper strain E. coli MT1694 could successfully mobilize pUO-SeVR1 from Salmonella Enteritidis CNM4839/03 into E. coli K12 JS3. In this way, a transconjugant that carried pUO-SeVR1 alone (Te-4839), was obtained.

Southern blot hybridizations (Figure S1) and PCR experiments using described primers,2–6 or newly designed ones (forward/ reverse sequences in brackets), demonstrated the presence on pUO-SeVR1 of IncFII and IncFIB (CTCCGGCAGTAACCGAGATG/ GCCCTGACTTCTTAAAGG) replicons, and the spvR (TCGACGCTG-GAAAGAGAGC/CTGCGAGTGCTGATCTCTG), spvA, spvB, spvC, rsk, rck, para, paraB, mig-5 (GAGGAGGT/CGAGGATGACT/CCGAC-CATCGACAAAATCA), srgB (ACCTTCACCAACAGTTC/CCGTTGTT CCGTCTATACTG), srgC (TTCTGGCAGGAGATC/CCCGTCTTGA AGCGGCTATGCG), pefA, pefB, pefC and pefD genes, all characteristic of pSEV, as well as the absence of pef–orf7, but not the entire pef–orf7–srgA region in the hybrid plasmid (Figure S1a), which could not be detected by Southern blot hybridizations (Figure S1b).

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The genetic context of the R genes was established by cloning and sequencing experiments.7 A class 1 integron with the 700 bp/dfrA7 variable region was carried by Tn5086 from the Tn21 subfamily. In pUO-SeVR1 (Figure 1a), this transposon appeared to be defective, since we failed to detect the tnpA gene and the mer operon. Upstream of the integron, the tnpR gene was truncated by insertion of IS26, which also disrupted the next orf (orf-E1; 99% identity with PSL7033 of PSL, which encodes a putative inner membrane protein; accession no. AE007647). Downstream of dfrA7, the 3’CS segment of the integron contained an orf5 deleted by insertion of a second copy of IS26, which is followed by orf1 (unknown function), catA2, IS4321 and a third copy of IS26. Downstream, a defective Tn1721 transposon was located, carrying the characteristic genes tetR, tet(A), pesc and tnpA2, but not the entire tnpA gene.

The blaTEM1 gene of pUO-SeVR1 was associated with Tn3 (Figure 1b), and this element has disrupted a gene that encodes a protein 60% identical to the product of mucB. This gene is homologous to E. coli umuC, which encodes a DNA polymerase specialized in translesion replication, a process activated by the SOS response under stress conditions imposed by DNA damage.8 In pUO-SeVR1, mucB has suffered a deletion