Methicillin-susceptible Staphylococcus aureus ST398-t571 harbouring the macrolide–lincosamide–streptogramin B resistance gene  
\( \text{erm}(T) \) in Belgian hospitals

Stien Vandendriessche\(^1,2\)*, Kristina Kadlec\(^3\), Stefan Schwarz\(^3\) and Olivier Denis\(^1\)

\(^1\)Laboratoire de Référence MRSA-Staphylocoques, Service de Microbiologie, Université Libre de Bruxelles, Hôpital Erasme, Brussels, Belgium; \(^2\)Veterinary and Agrochemical Research Center (VAR), Brussels, Belgium; \(^3\)Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany

*Corresponding author. Service de Microbiologie, Hôpital Erasme, 1070 Brussels, Belgium. Tel: +32-2-555-69-71; Fax: +32-2-555-31-10; E-mail: stien.vandendriessche@ulb.ac.be

Received 19 May 2011; returned 23 June 2011; revised 25 July 2011; accepted 27 July 2011

Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA), collected from 109 Belgian acute-care hospitals during a national survey in 2008, were investigated for macrolide–lincosamide (ML) resistance with particular emphasis on the analysis of  \( \text{erm}(T) \)-carrying isolates.

Methods: In total, 314 MRSA and 212 MSSA were collected and characterized by spa typing. The SCCmec type of MRSA was determined. Resistance to ML antibiotics was detected by agar dilution and resistant strains were screened by PCR for  \( \text{erm}(A) \),  \( \text{erm}(C) \) and  \( \text{msr}(A) \). Five ML-resistant MSSA isolates, negative by PCR for the aforementioned genes, were further characterized.

Results: Half of all MRSA isolates (\( \text{n} = 157 \); 50.0%) were resistant to erythromycin and harboured the gene  \( \text{erm}(A) \) (\( \text{n} = 112 \)),  \( \text{erm}(C) \) (\( \text{n} = 41 \)),  \( \text{erm}(A) + \text{erm}(C) \) (\( \text{n} = 3 \)) or  \( \text{msr}(A) \) (\( \text{n} = 1 \)). The  \( \text{erm}(A) \) gene was mainly present in MRSA spa-CC002-ST5-SCCmec II and spa-CC008-ST8-SCCmec IV (where CC stands for clonal complex and ST stands for sequence type); the distribution of  \( \text{erm}(C) \) was more diverse. Thirty-five of the 40 erythromycin-resistant MSSA (18.9%) carried the gene  \( \text{erm}(A) \) (\( \text{n} = 17 \)),  \( \text{erm}(C) \) (\( \text{n} = 9 \)) or  \( \text{msr}(A) \) (\( \text{n} = 9 \)). The remaining five MSSA were ST398-t571 isolates, which exhibited closely related ApaI PFGE patterns, harboured the gene  \( \text{erm}(T) \) in the chromosomal DNA and did not exhibit additional resistances. These isolates were from severe infections in patients, of whom four had no contact and one had only indirect contact with livestock via a family member working in animal husbandry.

Conclusions: The ML–streptogramin B (‘MLSB’) resistance genes  \( \text{erm}(A) \) or  \( \text{erm}(C) \) were detected in the majority of ML-resistant MRSA and MSSA isolates. The  \( \text{erm}(T) \) gene was identified in MSSA ST398 isolates from five independent patients who lacked direct contact with livestock.

Keywords: animal reservoirs, MLS\(_B\), S. aureus, surveillance

Introduction

In Staphylococcus aureus, three main types of mechanisms are responsible for acquired resistance to macrolide, lincosamide and streptogramin antibiotics: target site modification, active efflux and drug modification.\(^1\) The predominant mechanism of resistance is methylation of the A2058/A2059 residues in domain V of 23S rRNA. The respective methylases are encoded by  \( \text{erm} \) genes. Among the nine different  \( \text{erm} \) genes currently known to occur in the genus Staphylococcus (http://faculty.washington.edu/marilynr/ermweb4.pdf),  \( \text{erm}(A) \) and  \( \text{erm}(C) \) are most frequent.\(^1\) The  \( \text{erm} \) genes confer cross-resistance to macrolides, lincosamides and streptogramin B (MLS\(_B\)) antibiotics and can be expressed constitutively or inducibly.\(^1\) Resistance through active efflux in Staphylococcus spp. is mostly mediated by the ABC transporter  \( \text{Msr}(A) \), which confers resistance to macrolides and streptogramin B antibiotics. Drug modification by inactivating enzymes, such as  \( \text{Mph}(C) \) or  \( \text{Lnu}(A) \), has also been reported.\(^1\)\(^2\) These two enzymes confer resistance to macrolides and lincosamides, respectively.

The  \( \text{erm}(T) \) gene has only recently been described in staphylococci, but has previously been found in streptococci, enterococci and lactobacilli.\(^3\) During the last 2 years, the gene has been identified in livestock-associated methicillin-resistant
S. aureus (MRSA) ST398 from swine, cattle and turkey meat (where ST stands for sequence type)1–5 and in methicillin-susceptible S. aureus (MSSA) ST398 isolated from healthy human carriers in Spain.6

Since 1992, the Belgian reference laboratory has conducted surveys every 2 years to monitor the evolution of genotypes and antimicrobial resistance profiles of MSSA and MRSA in acute care hospitals. The objectives of the current study were to describe the macrolide–lincosamide (ML) resistance patterns and the distribution of ML resistance genes among MRSA and MSSA collected in the 2008 survey. Particular emphasis was put on the analysis of isolates that carried the erm(T) gene.

Materials and methods

Bacterial strains

In 2008, 314 MRSA and 212 MSSA isolates were collected from 109 acute care hospitals during a national survey as previously described.1 Species identification and methicillin resistance were confirmed by nuc-16S rRNA-mecA multiplex PCR. All isolates were sent to the reference laboratory with a structured case report form describing demographic data (age and gender) and the source of the specimen.

Susceptibility testing and detection of ML resistance determinants

MICs of erythromycin and clindamycin were determined by the agar dilution method (test range 0.06–128 mg/L) as recommended by CLSI.2456 The distribution of the MIC values of clindamycin among the MRSA strains revealed a similar range, between 0.06 and ≥256 mg/L with MIC50 at 0.12 mg/L and MIC90 at ≥256 mg/L. Of the 314 MRSA strains tested, 157 (50.0%) were resistant to erythromycin and 110 of these (70.1%) were co-resistant to clindamycin. MRSA strains resistant to macrolides or macrolides/lincosamides harboured the gene erm(A) (n = 112), erm(C) (n = 41), erm(A) and erm(C) (n = 3) or msr(A) (n = 1) (Table 1). While 101 (87.8%) of the 115 erm(A)-positive strains expressed this gene constitutively, an inducible ML resistance phenotype was detected in 32 (72.7%) of the 44 strains that carried an erm(C) gene. By molecular typing, almost 90% of the MRSA strains were assigned to one of the following four clones: spa-CC008-ST5-SCCmec IV (39.2%), spa-CC008-ST8-SCCmec IV (28.9%), spa-CC002-ST5-SCCmec II (12.4%) and spa-CC002-ST5-SCCmec IV (7.3%). For MRSA, 38 (97.4%) of the spa-CC002-ST5-SCCmec II isolates and 64 (70.3%) of the spa-CC008-ST8-SCCmec IV isolates harboured the erm(A) gene (Table 1). It should be noted that the erm(A)-carrying transposon Tn554 is commonly found in SCCmec cassettes of type II, whereas the presence of the erm(A) gene is variable in MRSA ST8-SCCmec IV isolates.12 In contrast, the erm(C) gene was more widely distributed among different MRSA clones (Table 1).

The distribution of the MIC values of erythromycin among the MSSA strains ranged between 0.06 and ≥256 mg/L with MIC50 at 0.25 mg/L and MIC90 at 128 mg/L. The distribution of the MIC values of clindamycin among the MSSA strains ranged between 0.06 and ≥256 mg/L with MIC50 at 0.06 mg/L and MIC90 at 0.25 mg/L. Forty (18.9%) of the 212 MSSA isolates were resistant to erythromycin, seven of which (17.5%) were co-resistant to clindamycin. The genes erm(A) (n = 17), erm(C) (n = 9) or msr(A) (n = 9) were detected in 35 of the 40 isolates (Table 1), while the remaining five MSSA isolates did not harbour any of these three resistance genes. Further analysis revealed the presence of the erm(T) gene in all five isolates. The MSSA isolates showed a wider genotypic diversity than the MRSA isolates, with 17 spa-CCs detected. However, approximately 40% of the MSSA isolates clustered in the four spa-CCs that were also detected among the MRSA isolates: spa-CC002 (14.6%), spa-CC008 (8.5%), spa-CC032 (1.4%) and spa-CC038 (15.1%). The distribution of erm and msr(A) genes in MSSA isolates was not associated with specific genotypes (Table 1).

Epidemiological and clinical background of the patients

For the five patients from whom an erm(T)-positive MSSA was isolated, additional data on whether the patients were in contact with livestock prior to hospitalization, whether they lived in a pig-dense area and whether the MSSA isolates provoked infection were collected using a retrospective questionnaire.

Results and discussion

Five erythromycin-resistant but clindamycin-susceptible MSSA strains, which were negative by PCR for erm(A), erm(C) and msr(A), were further characterized. To check for inducible resistance to clindamycin, the D-zone test was performed as recommended in the CLSI document M100-S20.8 The presence of the erm(B) or erm(T) gene was investigated by PCR and the amplicons obtained were sequenced for confirmation.8,4 To examine the location of the detected resistance gene, plasmid profiling and Southern blot analysis were performed as previously described.3 Southern blot analysis was performed on whole-cell DNA digested with EcoRI, EcoRV, PstI, BglII, HindIII, PvuII, XbaI or NdeI. The five MSSA strains were genotyped by PFGE as described previously, using the enzymes SmaI and ApaI.6,7 One isolate was further genotyped by MLST.11 Panton–Valentine leucocidin (PVL)-encoding genes were tested by PCR.7 Characterization of ML-resistant strains with an unknown resistance determinant

Five erythromycin-resistant but clindamycin-susceptible MSSA strains, which were negative by PCR for erm(A), erm(C) and msr(A), were further characterized. To check for inducible resistance to clindamycin, the D-zone test was performed as recommended in the CLSI document M100-S20.8 The presence of the erm(B) or erm(T) gene was investigated by PCR and the amplicons obtained were sequenced for confirmation.8,4 To examine the location of the detected resistance gene, plasmid profiling and Southern blot analysis were performed as previously described.3 Southern blot analysis was performed on whole-cell DNA digested with EcoRI, EcoRV, PstI, BglII, HindIII, PvuII, XbaI or NdeI. The five MSSA strains were genotyped by PFGE as described previously, using the enzymes SmaI and ApaI.6,7 One isolate was further genotyped by MLST.11 Panton–Valentine leucocidin (PVL)-encoding genes were tested by PCR.7

The presence of the D-zone test was performed as recommended in the CLSI document M100-S20.8 The presence of the erm(B) or erm(T) gene was investigated by PCR and the amplicons obtained were sequenced for confirmation.8,4 To examine the location of the detected resistance gene, plasmid profiling and Southern blot analysis were performed as previously described.3 Southern blot analysis was performed on whole-cell DNA digested with EcoRI, EcoRV, PstI, BglII, HindIII, PvuII, XbaI or NdeI. The five MSSA strains were genotyped by PFGE as described previously, using the enzymes SmaI and ApaI.6,7 One isolate was further genotyped by MLST.11 Panton–Valentine leucocidin (PVL)-encoding genes were tested by PCR.7

Epidemiological and clinical background of the patients

For the five patients from whom an erm(T)-positive MSSA was isolated, additional data on whether the patients were in contact with livestock prior to hospitalization, whether they lived in a pig-dense area and whether the MSSA isolates provoked infection were collected using a retrospective questionnaire.

Results and discussion

The distribution of the MIC values of erythromycin among the MRSA strains ranged between 0.06 and ≥256 mg/L with MIC50 at 0.5 mg/L and MIC90 at ≥256 mg/L. The distribution of the MIC values of clindamycin among the MRSA strains revealed a similar range, between 0.06 and ≥256 mg/L with MIC50 at 0.12 mg/L and MIC90 at ≥256 mg/L. Of the 314 MRSA strains tested, 157 (50.0%) were resistant to erythromycin and 110 of these (70.1%) were co-resistant to clindamycin. MRSA strains resistant to macrolides or macrolides/lincosamides harboured the gene erm(A) (n = 112), erm(C) (n = 41), erm(A) and erm(C) (n = 3) or msr(A) (n = 1) (Table 1). While 101 (87.8%) of the 115 erm(A)-positive strains expressed this gene constitutively, an inducible ML resistance phenotype was detected in 32 (72.7%) of the 44 strains that carried an erm(C) gene. By molecular typing, almost 90% of the MRSA strains were assigned to one of the following four clones: spa-CC008-ST5-SCCmec IV (39.2%), spa-CC008-ST8-SCCmec IV (28.9%), spa-CC002-ST5-SCCmec II (12.4%) and spa-CC002-ST5-SCCmec IV (7.3%). For MRSA, 38 (97.4%) of the spa-CC002-ST5-SCCmec II isolates and 64 (70.3%) of the spa-CC008-ST8-SCCmec IV isolates harboured the erm(A) gene (Table 1). It should be noted that the erm(A)-carrying transposon Tn554 is commonly found in SCCmec cassettes of type II, whereas the presence of the erm(A) gene is variable in MRSA ST8-SCCmec IV isolates.12 In contrast, the erm(C) gene was more widely distributed among different MRSA clones (Table 1).

The distribution of the MIC values of erythromycin among the MSSA strains ranged between 0.06 and ≥256 mg/L with MIC50 at 0.25 mg/L and MIC90 at 128 mg/L. The distribution of the MIC values of clindamycin among the MSSA strains ranged between 0.06 and ≥256 mg/L with MIC50 at 0.06 mg/L and MIC90 at 0.25 mg/L. Forty (18.9%) of the 212 MSSA isolates were resistant to erythromycin, seven of which (17.5%) were co-resistant to clindamycin. The genes erm(A) (n = 17), erm(C) (n = 9) or msr(A) (n = 9) were detected in 35 of the 40 isolates (Table 1), while the remaining five MSSA isolates did not harbour any of these three resistance genes. Further analysis revealed the presence of the erm(T) gene in all five isolates. The MSSA isolates showed a wider genotypic diversity than the MRSA isolates, with 17 spa-CCs detected. However, approximately 40% of the MSSA isolates clustered in the four spa-CCs that were also detected among the MRSA isolates: spa-CC002 (14.6%), spa-CC008 (8.5%), spa-CC032 (1.4%) and spa-CC038 (15.1%). The distribution of erm and msr(A) genes in MSSA isolates was not associated with specific genotypes (Table 1).
Nucleotide sequence analysis of the \(\text{erm}(T)\) PCR amplicons indicated 98% identity to the \(\text{erm}(T)\) gene described on plasmid pKKS25 from a porcine MRSA ST398 strain. Only one of five MSSA isolates in this study harboured a plasmid, which, however, did not carry the \(\text{erm}(T)\) gene. The chromosomal location of \(\text{erm}(T)\) was confirmed by Southern blot analysis performed with various restriction endonucleases. The hybridization patterns suggested that the \(\text{erm}(T)\) gene was located on the chromosome in three copies (Figure 1a).

All five MSSA strains had spa type t571 and were non-typeable by SmaI-PFGE. ApaI macrorestriction analysis revealed indistinguishable or closely related fragment patterns, which suggests a clonal background (Figure 1b). By MLST, the five strains belonged to ST398, which is commonly found in livestock and persons in close contact with ST398-positive animals.3,12 Interestingly, the five MSSA ST398 isolates found in this study originated from patients hospitalized in unrelated acute-care hospitals, which were located in different geographical regions of Belgium. None of the patients lived in a pig-dense area, and only one patient had indirect contact with livestock, through his daughter who worked on a farm (Table 2).

In a large-scale European study conducted in 2006–07, MSSA ST398 represented 1.3% of MSSA isolates causing invasive infections.13 Since then, other severe infections by MSSA ST398 have been reported from Europe and China.14–17 Interestingly, the majority of the isolates described in these reports also belonged to spa type t57114–17 and some even carried PVL genes.15,17 In this study, none of the MSSA ST398 strains harboured PVL genes.

The strains were isolated from blood \((n=1)\), respiratory tract infections \((n=2)\) or wounds \((n=2)\). Two isolates were collected from patients with severe infections, one from a 48-year-old woman with peritonitis and subsequent bacteraemia and one from a 67-year-old woman with pneumonia (Table 2).

D-zone testing suggested that the \(\text{erm}(T)\) was inducibly expressed in the five MSSA isolates from this study. In contrast, the \(\text{erm}(T)\) gene that has recently been detected in a porcine MRSA ST398 was constitutively expressed and resided on a plasmid that also harboured the resistance genes \(\text{tet}(L)\) and \(\text{dfrK}\), encoding resistance to tetracyclines and trimethoprim, respectively.3 Tetrandocline resistance is a common feature of MRSA ST398 strains,1,4,12 as tetracyclines are among the antimicrobial agents most frequently used in farm animals in most European countries,18 including Belgium (http://www.belvetSAC.ugent.be/pages/home/BelvetSAC_report_2007-8-9%20finaal.pdf). The MSSA ST398 isolates detected in this study, however, were

---

Table 1. Distribution of resistance genes among ML-resistant MRSA and MSSA strains according to spa-CC (and, if available, SCCmec type)

<table>
<thead>
<tr>
<th>MRSA/MSSA</th>
<th>Clones</th>
<th>Number of strains</th>
<th>Strains resistant to</th>
<th>Strains harbouring resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>erythromycin</td>
<td>clindamycin</td>
</tr>
<tr>
<td>MRSA</td>
<td>spa-CC002-SCCmec I(^{b})</td>
<td>39</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>spa-CC002-SCCmec IV(^{b})</td>
<td>23</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>spa-CC008-SCCmec IV(^{b})</td>
<td>91</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>spa-CC032-SCCmec IV</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>spa-CC038-SCCmec IV(^{b})</td>
<td>123</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>31</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>314</td>
<td>157</td>
<td>110</td>
</tr>
<tr>
<td>MSSA</td>
<td>spa-CC002</td>
<td>31</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>spa-CC008</td>
<td>18</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>spa-CC032</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>spa-CC038</td>
<td>32</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>128</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>212</td>
<td>40</td>
<td>7</td>
</tr>
</tbody>
</table>

---

\(^{a}\)The presence of the \(\text{erm}(T)\) gene was tested for five MSSA ST398 isolates only.

\(^{b}\)Including MRSA with variants of SCCmec that harbour two ccr complexes.

Figure 1. (a) \(\text{erm}(T)\) hybridization patterns obtained from EcoRI-digested whole-cell DNA and (b) macrorestriction patterns obtained with the restriction endonucleases ApaI and SmaI. A–E, MSSA isolates A–E; M, size markers [in (a) λ-DNA HindIII digest, DNA molecular weight marker II, Roche; in (b) SmaI digest of whole-cell DNA of \(S.\) \(\text{aureus}\) 8325]; +, positive control [\(\text{erm}(T)\) amplicons of 536 bp]; –, negative control.
susceptible to all additional antimicrobial agents tested. The absence of tetracycline resistance was also observed in some of the other previously reported virulent MSSA ST398 isolates.\(^{15-17}\)

In addition, the five MSSA isolates harboured the downstream common sequence (dcs) of SCC\(\text{mec}\) and the insertion sequence IS\(\text{1272}\) was detected in the bloodstream isolate (data not shown). The presence of remnants of SCC\(\text{mec}\) was previously reported for MSSA ST398 from bacteraemia\(^{16}\) and might suggest that the strains were formerly MRSA. Further research is necessary to examine whether the MSSA ST398 strains isolated from hospitalized patients in this study are related to the MRSA ST398 strains commonly detected in farm animals.

Bacteria in livestock constitute a large reservoir of resistance genes.\(^{19}\) Seen from the healthcare perspective, one of the major concerns regarding methicillin-resistant staphylococci in livestock is that they could act as a reservoir of resistance genes and transfer these genes to human-adopted healthcare- or community-associated MRSA clones. One example of a resistance gene that was initially detected in animal staphylococci is the cfr gene, encoding resistance to members of six different classes of antimicrobial agents, including oxazolidinones, such as linezolid. Recently, the cfr gene was detected in a PVL-positive CA-MRSA USA300 strain isolated from a human patient.\(^{20}\)

Whereas the erm(T) gene was originally detected in lactobacilli, streptococci and enterococci,\(^{1}\) it has recently been detected in MRSA ST398 isolated from swine,\(^{2}\) cattle\(^{3}\) and turkey meat,\(^{18}\) and in MSSA ST398 from healthy humans\(^{4}\) and, as shown in this study, also from diseased humans. The question of whether the erm(T) gene in human MSSA ST398 originates from animal MRSA remains to be answered.

Table 2. Characteristics of the five patients carrying MSSA ST398

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Unit</th>
<th>Origin</th>
<th>Clinical background</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>62</td>
<td>M</td>
<td>other(^a)</td>
<td>respiratory tract</td>
<td>no data available</td>
</tr>
<tr>
<td>B</td>
<td>67</td>
<td>F</td>
<td>ICU</td>
<td>respiratory tract</td>
<td>pneumonia</td>
</tr>
<tr>
<td>C</td>
<td>48</td>
<td>F</td>
<td>ICU</td>
<td>blood culture</td>
<td>peritonitis, subsequent bacteraemia</td>
</tr>
<tr>
<td>D</td>
<td>75</td>
<td>F</td>
<td>medical ward</td>
<td>wound</td>
<td>asymptomatic carriage</td>
</tr>
<tr>
<td>E(^b)</td>
<td>74</td>
<td>M</td>
<td>medical ward</td>
<td>wound</td>
<td>asymptomatic carriage</td>
</tr>
</tbody>
</table>

M, male; F, female; ICU, intensive care unit.

\(^a\)Not isolated from a patient hospitalized in an ICU or in a medical, geriatric or surgical ward.

\(^b\)This person had indirect contact with livestock.

References

10. Kondo Y, Ito T, Ma XX et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid


