Detection of the novel vga(E) gene in methicillin-resistant Staphylococcus aureus CC398 isolates from cattle and poultry

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Sir,

The genes vga(A) and vga(C)\(^1\) and the most recently identified gene, vga(E),\(^2\) as well as the gene cfr, mediate transferable resistance to pleuromutilins in staphylococci.\(^3\) The vga genes encode ABC transporters, which also export streptogramin A antibiotics and lincosamides, and the cfr gene encodes a methyltransferase that confers additional resistance to penicillins, linco-

samides, oxazolidinones and streptogramin A antibiotics. All these genes are located on either plasmids or transposons. Point mutations in the domain V of 23S rRNA or in the rplC gene, encoding the ribosomal protein L3, are also known to mediate pleuromutilin resistance in staphylococci.\(^5\)

In two previous studies on methicillin-resistant Staphylococcus aureus (MRSA) in dairy cattle (n = 25)\(^6\) and in poultry meat and poultry meat products (n = 32)\(^7\) and one ongoing study on MRSA from cattle (n = 11) and poultry (n = 2) collected in the GERM-Vet programme 2008–09, we detected pleuromutilin resistance in a total of nine MRSA isolates, which were negative by PCR for the cfr gene and the staphylococcal genes vga(A), vga(B) and vga(C), but also for the enterococcal gene vga(D) and the streptococcal gene isa(C).\(^7\) Moreover, point mutations in the 23S rRNA and rplC genes of these isolates were not detected. These nine isolates originated from fresh chicken and turkey meat, turkey meat products,\(^6\) cattle with bovine clinical mastitis\(^8\) and a turkey with an infection of the musculoskeletal system (Table 1). The aim of the present study was to investigate these nine isolates for the presence of the most recently identified gene, vga(E). This gene has so far been found only in MRSA ST398 (where ST stands for sequence type) isolates of porcine origin in Switzerland.\(^2\)

If not already done in previous studies, the MRSA isolates were subjected to spa, SCCmec and dru typing as well as two CC398-specific PCRs as described previously.\(^9\) All nine isolates were assigned to the clonal complex (CC) 398, and all but one shared SCCmec type V and showed spa type t034. One isolate from turkey meat had SCCmec type IV and showed spa type t011. Four different dru types were detected, with dru type dt6j found in six isolates and dru types dt10q, dt6m and dt11a in single isolates (Table 1).

Despite the different origin of the isolates, susceptibility testing by broth microdilution following the recommendations given in the CLSI documents M31-A3\(^9\) and M100-S21\(^10\) revealed rather uniform susceptibility patterns, which included resistance to ß-lactams, tetracyclines, trimethoprim, MLS\(_B\) antibiotics, spectinomycin and tiamulin. The nine isolates differed only slightly in their classification as resistant or intermediate to quinupristin/dalfopris-
tin (Table 1). All nine isolates carried mecA and the ß-lactamase operon blaZ-blaI-blaR. Tetracycline resistance was mediated by the gene tet(M), which was present either alone (n = 2) or in combination with tet(K) (n = 6) or with tet(K) + tet(L) (n = 1). The dihy-

Downloaded from https://academic.oup.com/jac/article-abstract/67/2/503/701137 by guest on 17 April 2019 drofolate reductase gene dfrK was detected in all but one of the trimethoprim-resistant isolates. One isolate carried the gene dfrS1 in addition to dfrK. The RNA methylase gene erm(A) was detected in all isolates, either alone (n = 3) or in combination with erm(B) (n = 4) or erm(C) (n = 2). The spectinomycin resistance gene spc was also detected in all nine isolates. The simultaneous presence of the genes erm(A) and spc suggested the presence of a Tn554-like transposon. This assumption was supported by the PCR-detected linkage of these two genes.\(^5\)

So far, the novel vga(E) gene has been identified in a limited number of porcine MRSA ST398-t034 in Switzerland.\(^2\) In these isolates, vga(E) proved to be part of the Tn554-like multidrug-resistance transposon Tn6133. This transposon consisted of a complete transposon, Tn554, in which a vga(E)-

containing DNA segment of 4787 bp was integrated between the erm(A) gene and a Tn554-associated reading frame (orf) of unknown function. PCR analysis of whole-cell DNA with primers specific for the vga(E) gene demonstrated that this novel pleurom-

mutilin, lincosamide and streptogramin A resistance gene was present in the nine isolates of cattle and poultry origin collected in Germany. Sequence analysis of the vga(E) amplicons of two randomly chosen isolates (one from cattle and one from poultry) confirmed the specificity of the amplicons. To investigate whether a Tn6133 transposon was also present in the nine isolates of this study, two PCR assays were designed to prove the linkage of the vga(E) gene with its upstream and downstream regions.\(^7\) One primer pair specific for the 5′ end of vga(E) and the 3′ end of the Tn554-associated orf of unknown function (vgaE_fwd 5′-GAAATATGGATACAGCCAGTGAG-3′, vgaE_rev 5′-CAGTCTCTTTTGAAATTGAGACC-3′; amplicon size 1685 bp; annealing temperature 52°C) and the other primer pair specific for the erm(A) regulatory region and the 3′ end of vga(E) (ermA_fwd 5′-CATAGCTCTTTTGCTAAATGTC-3′, vgaE_rev 5′-CAGTCTCTTTTGAAATTGAGACC-3′; amplicon size 4977 bp; annealing temperature 50°C) yielded...
Table 1. Characteristics of the nine MRSA CC398 isolates included in this study

<table>
<thead>
<tr>
<th>Origin</th>
<th>Isolate</th>
<th>SCCmec type</th>
<th>spa type</th>
<th>dru type</th>
<th>Resistance patterna,b</th>
<th>Resistance genesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken meat</td>
<td>Chi-3</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, (Q/D)</td>
<td>+ (K)+(M) K (A) + +</td>
</tr>
<tr>
<td>Turkey meat</td>
<td>Tur-10</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, (Q/D)</td>
<td>+ (K)+(M) K (A)+(B) + +</td>
</tr>
<tr>
<td></td>
<td>Tur-19</td>
<td>IV</td>
<td>t011</td>
<td>dt10q</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (K)+(M) SI+K (A)+(C) + +</td>
</tr>
<tr>
<td></td>
<td>Tur-22</td>
<td>V</td>
<td>t034</td>
<td>dt6m</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (M) K (A)+(C) + +</td>
</tr>
<tr>
<td>Turkey meat products</td>
<td>Tur-6</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (K)+(M) K (A) + +</td>
</tr>
<tr>
<td></td>
<td>Tur-8</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (K)+(M) K (A)+(B) + +</td>
</tr>
<tr>
<td>Turkeyd</td>
<td>81365</td>
<td>V</td>
<td>t034</td>
<td>dt11a</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (M) K (A)+(B) + +</td>
</tr>
<tr>
<td>Cattled</td>
<td>Rd-51</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (K)+(L)+(M) K (A)+(B) + +</td>
</tr>
<tr>
<td></td>
<td>Rd-53</td>
<td>V</td>
<td>t034</td>
<td>dt6j</td>
<td>BLA, TET, TMP, MLSb, SPC, TIA, Q/D</td>
<td>+ (K)+(M) — (A) + +</td>
</tr>
</tbody>
</table>

aBLA, β-lactam antibiotics; MLSb, macrolides/lincosamides/streptogramin B; Q/D, quinupristin/dalfopristin; (Q/D), intermediate to quinupristin/ dalfopristin based on MIC; SPC, spectinomycin; TET, tetracyclines; TIA, tiamulin; TMP, trimethoprim.

bDespite the lack of CLSI-approved breakpoints, isolates that showed high MIC values of TMP (≥256 mg/L), SPC (≥512 mg/L) and TIA (≥16 mg/L) were considered to be resistant.

cThe different subtypes or combinations of subtypes of tet, dfr and erm genes present are indicated by the corresponding letters.

dClinical isolates.

fragments of the expected sizes in all nine isolates. These data, in combination with the proved linkage of erm(A) and spc, strongly suggest that the vga(E) gene is also located on a Tn6133 transposon in the bovine and avian MRSA CC398 isolates of this study.

The results of this study showed that the resistance gene vga(E) is present not only in porcine MRSA but also in MRSA from food and food products of poultry origin, as well as in clinical isolates from cattle and turkey. The location of vga(E) on a transposon might facilitate its dissemination.

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Transparency declarations

None to declare.

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