Veterinary Microbiology 191 (2016) 88–95

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Heavy metal and disinfectant resistance genes among livestock-associated methicillin-resistant *Staphylococcus aureus* isolates

M. Angeles Argudín[a,b,1], Birgit Lauzat[a], Britta Kraushaar[a], Patricia Alba[c], Yvonne Agerso[d], Lina Cavaco[d], Patrick Butaye[e,f], M. Concepción Porrero[g], Antonio Battisti[c], Bernd-Alois Tenhagen[a], Alexandra Fetsch[a], Beatriz Guerra[a,*]

[a] Department of Biological Safety, Federal Institute for Risk Assessment (BfR), Max-Dohrn-Straße 8-10, Berlin, Germany
[b] Department of Bacterial Diseases, Veterinary and Agrochemical Research Centre (CODA-CERVA), Brussels, Groeselenbergstraat 99, B-1180 Ukkel, Belgium
[c] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT), Via Appia Nuova 1411, 00178, Rome, Italy
[d] Technical University of Denmark (DTU), National Food Institute, Research group for Genomic Epidemiology, Søltofts Plads Building 221, 2800 Lyngby, Denmark
[e] Department of Biomedical Sciences, Ross University, P.O. Box 334, Basseterre, St Kitts, West Indies
[f] Department of Pathology, Bacteriology, and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[g] Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense Madrid (UCM), Madrid, Spain

ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 30 May 2016
Accepted 8 June 2016

Keywords:
Zinc
Copper
Arsenic compounds
Cadmium
SCC*mec*

ABSTRACT

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has emerged in animal production worldwide. Most LA-MRSA in Europe belong to the clonal complex (CC) 398. The reason for the LA-MRSA emergence is not fully understood. Besides antimicrobial agents used for therapy, other substances with antimicrobial activity applied in animal feed, including metal-containing compounds might contribute to their selection. Some of these genes have been found in various novel SCC*mec* cassettes. The aim of this study was to assess the occurrence of metal-resistance genes among a LA-*S. aureus* collection [n = 554, including 542 MRSA and 12 methicillin-susceptible *S. aureus* (MSSA)] isolated from livestock and food thereof. Most LA-MRSA isolates (76%) carried at least one metal-resistance gene. Among the LA-MRSA CC398 isolates (n = 456), 4.8%, 0.2%, 24.3% and 71.5% were positive for *arsA* (arsenic compounds), *cadD* (cadmium), *copB* (copper) and *czrC* (zinc/cadmium) resistance genes, respectively. In contrast, among the LA-MRSA non-CC398 isolates (n = 86), 1.2%, 18.6% and 16.3% were positive for the *cadD*, *copB* and *czrC* genes, respectively, and none were positive for *arsA*. Of the LA-MRSA CC398 isolates, 72% carried one metal-resistance gene, and the remaining harboured two or more in different combinations. Differences between LA-MRSA CC398 and non-CC398 were statistically significant for *arsA* and *czrC*. The *czrC* gene was almost exclusively found (98%) in the presence of SCC*mec* V in both CC398 and non-CC398 LA-MRSA isolates from different sources. Regarding the LA-MSSA isolates (n = 12), some (n = 4) were also positive for metal-resistance genes. This study shows that genes potentially conferring metal-resistance are frequently present in LA-MRSA.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a zoonotic pathogen in animal production worldwide. Most of the livestock-associated (LA) MRSA in Europe belong to the clonal complex (CC) 398, although other CCs have also been described (Crombé et al., 2013; Butaye et al., 2016). The reason why LA-MRSA emerged is not fully understood. It has been suggested that tetracycline-resistance could have driven selection of MRSA CC398 (Crombé et al., 2013; Butaye et al., 2016). However,

* Corresponding author. Present address: European Food Safety Authority, Via Carlo Magno 1A, 43126 Parma, Italy.
E-mail address: beatriz.guerra@efsa.europa.eu (B. Guerra).
[1] Present address: Laboratoire de Référence MRSA-Staphylocoques, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium.

http://dx.doi.org/10.1016/j.vetmic.2016.06.004
0378-1135/

tetracycline-resistance is also common to methicillin-susceptible *S. aureus* (MSSA) of this lineage in livestock suggesting the presence of other selective mechanisms (Aarestrup et al., 2010). Besides antimicrobial agents used for therapy, other substances with antimicrobial or growth-promoting activity are used in food-animal productions. These include metal-containing compounds added as feed supplements. For instance, zinc-resistance (MIC > 2 mM) is highly prevalent in LA-MRSA CC398 strains from pigs, veal calves and humans (Cavaco et al., 2010, 2011) as well as in MRSA isolates belonging to other CCs (CC1 and CC97) from pigs (Cavaco et al., 2011). High occurrence (~20%) of copper sulphate-resistant (MIC > 12 mM) MRSA isolates has also been reported in pigs (Cavaco et al., 2011). Metal-resistance genes have been found in various SCC*mec* cassettes (mobile genetic elements harbouring the *mecA* or *mecC* gene, responsible for methicillin-resistance). Particularly, some of these genes are part of SCC*mec* V (5C2&5)c, IX and X found in ST398 isolates (Li et al., 2011). The SCC*mec* IX and X include a cadmium-resistance operon *cadDX*, the copper-resistance gene *copB* and a complete or partial resistance operon against arsenic compounds (*arsABCDR*). The SCC*mec* V (5C2&5)c includes the zinc/cadmium-resistance gen *czrC*, as well as the tetracycline-resistance gene *tet*(K) located in a pT181 plasmid integrated in the cassette (Li et al., 2011). The *tet*(K) has been also described as plasmid located in *S. aureus* (Argudín et al., 2014). Some LA-MRSA ST398 isolates carry the tetracycline genes *tet*(K) and *tet*(L), but *tet*(M) is the most frequent tetracycline-resistant gene found among LA-MRSA CC398 (Argudín et al., 2011).

Besides metal compounds, quaternary ammonium compounds (QACs) are used for disinfection in animal husbandry. The QAC-resistance genes are typically located in integrons in Gram-negative bacteria, while in *S. aureus* they are usually related to plasmids carrying antimicrobial-resistance genes (Jaglic and Cervinkova, 2012; Argudín et al., 2014). However, the presence of class 1 integrons in clinical MRSA isolates has also been described (Xu et al., 2011).

The aim of this study was to assess the occurrence of representative metal and tetracycline-resistance genes generally present in SCC*mec* elements, as well as class 1 integrons and QAC-encoding genes in LA-*S. aureus*.

2. Materials and methods

2.1. Bacteria collection

A total of 554 epidemiologically unrelated *S. aureus* isolates were included in this study. Most of the isolates isolates (n = 542) were LA-MRSA (456 CC398 and 86 non-CC398 isolates) recovered from livestock animals and/or their food products [pig (n = 218), bovine (n = 118), broiler (n = 28), turkey (n = 178)], between 2004 and 2012. Twelve isolates were LA-MSSA (8 CC398 and 4 non-CC398) and were collected between 2005 and 2011 from pig (n = 9), cattle (n = 2) or turkey (n = 1) sources.

A total of 313 out of the 554 isolates were selected from the collection of the German National Reference Laboratory for coagulase positive staphylococci including *S. aureus* (NRL-Staph) at the Federal Institute for Risk Assessment (BfR). These 313 isolates included 263 LA-MRSA-CC398, 46 LA-MRSA non-CC398, two LA-MSSA-CC398 and two LA-MSSA-non-CC398. A subset of the NRL-Staph collection, including 99 German and five Dutch isolates, was previously studied for their molecular epidemiology, antimicrobial-resistance and the presence/absence of antimicrobial and virulence determinants (Argudín et al., 2010, 2011).

The remaining 241 out of the 554 isolates were selected within the "LA-MRSA: Methicillin-resistant *Staphylococcus aureus* lineages in primary productions: multi-host pathogen, spill-over and spill-back between animals and humans?" project (EMIDA ERA NET LA-MRSA, http://era-platform.eu/era-nets/emida/) from Germany [126 isolates, NRL-Staph], Italy [56 isolates, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT)], Spain [33 isolates, Universidad Complutense Madrid (UCM)], Belgium [14 isolates, Veterinary and Agrochemical Research centre (CODA-CERVA)], and Denmark [12 isolates, Technical University of Denmark (DTU)]. The isolates from the EMIDA collection were 193 LA-MRSA-CC398, 40 LA-MRSA non-CC398, six LA-MSSA-CC398 and two LA-MSSA-non-CC398.

The selection of isolates was carried out to cover a variety of livestock species and sources. Only one isolate per farm, slaughter batch and/or *spa*-/SCC*mec*-type was selected, to cover a broad range of CC398 and non-CC398 subtypes.

2.2. PCR-amplification, DNA-array analysis and sequencing

The isolates were confirmed as *S. aureus* at the NRL-Staph by multiplex PCR targeting the 16S rRNA gene, the *S. aureus*-specific nuclease gene *nuc*, and the resistance gene *mecA* in the case of methicillin-resistant isolates (Table S1). The *mecA*-negative isolates were further tested for the presence of *mecC* using previously described oligonucleotides (Table S1). The CC assignment was done on the basis of protein A (*spa*) sequence typing (http://spaserver.ridom.de/) and/or multi locus sequence typing (MLST) (http://saureus.mlst.net/). SCC*mec* typing was performed with the protocols of Zhang et al. (2005), Kondo et al. (2007) and/or through microarray analysis (Alere Technologies, GmbH, Jena, Germany). Molecular and SCC*mec* typing results of 99 German (NRL-Staph) and five Dutch isolates were previously published by Argudín et al. (2010). Molecular typing and microarray results of CC1 and CC97 Italian isolates have been recently published (Alba et al., 2015; Feltrin et al., 2015).

The 554 isolates were tested via PCR-amplification (Table S1) for the presence of selected genes including (i) the ATPase efflux gene *arsA* as representative gene of the resistance operon against arsenic compounds (*arsABCDR*) associated to SCC*mec* IX; (ii) the ATPase efflux gene (*cadD*) of the cadmium-resistance operon *cadDX* associated to SCC*mec* IX and X; (iii) the copper-resistance gene (*copB*) associated to SCC*mec* IX and X; and (iv) the zinc and cadmium-resistance gene (*czrC*) associated to SCC*mec* V (5C2&5)c. The isolates were also tested for the presence of the tetracycline-resistance gene *tet*(K), located in the pT181 plasmid integrated in SCC*mec* V (5C2&5)c (Li et al., 2011). The presence of the tetracycline-resistance gene *tet*(M) was based on Argudín et al. (2011) and/or microarray analysis (Alere Technologies, GmbH, Jena, Germany). Additionally, all the isolates were tested for the presence of the *qac* genes [(*qacA/B* and *qacC/D* (also named *smr*)], and integrons (with degenerate primers that detect *intI1*, *intI2* and *intI3* integrase genes) (Table S1).

2.3. Statistical analysis

Fisher's exact test was used to determine statistical significant differences (*p*-value) between proportions of certain genes in certain subgroups of the population. A binary character dendrogram of similarity showing the profiles of absence/presence of SCC*mec* types and genes was built by using the unweighted pair group method with arithmetic mean (UPGMA) in Bionumerics 7.5 (Applied Maths, Belgium).

3. Results

3.1. Occurrence of metal-resistance genes

The 23.8% of the LA-MRSA isolates (n = 129) analysed was negative for the metal-resistance genes tested (Table S2). These