First report of swine-associated methicillin-resistant Staphylococcus aureus ST398 in Lithuania
Ruzauskas, M.; Couto, N.; Belas, A.; Kliimien, I.; Siugzdiniene, R.; Pomba, C.

Published in:
Polish Journal of Veterinary Sciences

DOI:
10.2478/pjvs-2013-0018

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2013

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
First report of swine-associated methicillin-resistant Staphylococcus aureus ST398 in Lithuania

M. Ruzauskas1, N. Couto2, A. Belas2, I. Klimiene1, R. Siugzdiniene,1 C. Pomba2

1 Institute of Veterinary, Lithuanian University of Health Sciences, Tilzes g. 18, LT-47181, Kaunas, Lithuania
2 Laboratory of Antimicrobial and Biocide Resistance, Interdisciplinary Centre of Research in Animal Health, Faculty of Veterinary Medicine, Technical University of Lisbon, Av. da Universidade Tecnica, 1300-477 Lisboa, Portugal

Abstract

During 2011, 160 nasal samples were taken from pigs on 8 different farms in Lithuania. Four methicillin-resistant Staphylococcus aureus (MRSA) isolates were obtained. The isolates were ST398, spa type t011 and SCCmec V and none carried the lukF/lukS genes. Strains were resistant to tetracycline, attributed to tetK and tetM genes, and to erythromycin owing to the ermB gene. One MRSA strain was resistant to trimethoprim/sulfamethoxazole and carried the dfrK gene. This is the first report on the presence and characteristics of livestock-associated MRSA isolated from pigs in Lithuania.

Key words: Staphylococcus aureus; ST398; methicillin-resistance, pigs

Introduction

Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) sequence type (ST) 398 is recognized as an important colonizer of food animal species, including pigs, cows, poultry and horses, and has been reported in several countries in Europe and North America. The purpose of this study was to determine the presence of MRSA in pigs in Lithuania.

Materials and Methods

Samples used in this study

During 2011, a total of 160 nasal samples were taken from pigs on 8 farms with capacity from 5000 up to 50 000 animals in four different counties of Lithuania. Weaned-off piglets (10 from each farm) as well as finishing pigs (10 from each farm) were selected as two different age groups.

Correspondence to: M. Ruzauskas, email: ruzauskas@lva.lt, tel.: +370 61 515 240
Table 1. Molecular characteristics of the four MRSA isolates from pigs.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sample source</th>
<th>Origin</th>
<th>SCCmec</th>
<th>ST</th>
<th>spa</th>
<th>PFGE pattern</th>
<th>PVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT1</td>
<td>Nasal swab</td>
<td>Pig V</td>
<td>398</td>
<td>t011</td>
<td>A1</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>LT2</td>
<td>Nasal swab</td>
<td>Pig V</td>
<td>398</td>
<td>t011</td>
<td>A2</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>LT3</td>
<td>Nasal swab</td>
<td>Pig V</td>
<td>398</td>
<td>t011</td>
<td>B</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>LT4</td>
<td>Nasal swab</td>
<td>Pig V</td>
<td>398</td>
<td>t011</td>
<td>A1</td>
<td>neg</td>
<td></td>
</tr>
</tbody>
</table>

*a The definition of a PFGE cluster was based on a similarity cut-off value of 80% using the unweighted pair group method (UPGMA).

Table 2. Antimicrobial resistance patterns and resistance genes identified in MRSA strains.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Resistance patterns</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT1</td>
<td>OXA, CLI, ERY, Q/D, TET</td>
<td>tetM, tetK, ermB</td>
</tr>
<tr>
<td>LT2</td>
<td>OXA, CLI, ERY, Q/D, TET, SXT</td>
<td>tetM, tetK, ermB, dfrK</td>
</tr>
<tr>
<td>LT3</td>
<td>OXA, CLI, ERY, Q/D, TET</td>
<td>tetM, tetK, ermB</td>
</tr>
<tr>
<td>LT4</td>
<td>OXA, CLI, ERY, Q/D, TET</td>
<td>tetM, tetK, ermB</td>
</tr>
</tbody>
</table>

CLI, clindamycin; ERY, erythromycin; OXA, oxacillin; Q/D, quinupristin/dalfopristin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole. All isolates were susceptible to ciprofloxacin, chloramphenicol, gentamicin, linezolid and vancomycin.

MRSA isolation and typing

MRSA isolation was performed using Mueller-Hinton Broth (Oxoid, UK) with 6.5% NaCl and Brilliance MRSA 2 Agar (Oxoid, UK). MRSA confirmation was obtained through detection of the *mecA* and *S. aureus* specific-*nuc* genes. Detection of sequence type 398, SCCmec and *spa* types were performed as described previously (Pomba et al. 2010, van Wamel et al. 2010). Clonality was assessed by Pulsed Field Gel Electrophoresis (PFGE) with Cfr9I restriction and isolates were also tested for the *lukF/lukS* genes encoding Panton-Valentine leukocidin (PVL) (Pomba et al. 2010).

Antimicrobial testing

Minimum Inhibitory Concentrations (MICs) of several antimicrobials were determined by broth microdilution (MicroScan PM21; Dade Behring, Deerfield, IL, USA) (Table 2) and interpreted according to CLSI guidelines M31-A3 and M100-S20.

Detection of genes encoding antimicrobial resistance

The presence of *tetK*, *tetM*, *ermA*, *ermB*, *ermC*, *vgaA*, *vgaC* and *dfrK* genes was studied by standard PCR protocols.

Results and Discussion

From a total of 160 samples tested, four MRSA strains were isolated (2.5%). All isolates were obtained from the same farm containing about 50 000 pigs located at the central part of the country. The MRSA isolates were identified as ST398, *spa* type t011 and SCCmec V. None of the MRSA isolates carried the PVL encoding genes (Table 1). Susceptibility testing revealed resistance to tetracycline, erythromycin and clindamycin in all MRSA (Table 2). Isolates were also resistant to the quinupristin/dalfopristin combination, but did not harbour the *vgaA* or *vgaC* genes coding for streptogramin A ABC transporters. One MRSA strain was resistant to trimethoprim/sulfamethoxazole and carried the novel resistance gene *dfrK*.

MRSA isolates obtained from Lithuanian pigs shared exactly the same features (ST398 and spa type t011) as those more frequently reported in the analysis of the baseline survey on the prevalence of MRSA in holdings of breeding pigs in the EU (EFSA 2009). MRSA isolates from Lithuania had also the same SCCmec V type and the absence of the PVL encoding genes as for previously described MRSA ST398 isolates from Belgium, Denmark, Germany, the Netherlands and Portugal. Still, one MRSA strain belonged to a different PFGE cluster and strains showed slightly different antimicrobial resistance patterns (Table 1). This might indicate that genetic variation continues to occur as a dynamic adaptation process within the pig
reservoir. To the best of our knowledge this is the first report on MRSA detection in livestock animals in Lithuania and also the report on LA-MRSA ST398 in pigs in Baltic countries. There is no scientific information regarding spread of MRSA ST398 in two neighbouring countries of Lithuania – Russia and Belarus. However, MRSA ST398 has been found in breeding pigs in Poland (EFSA 2009) as well as isolated from veterinarians in this country (Marszalek et al. 2009). The fast dissemination of this LA-MRSA ST398 meticillin-resistant European sub-lineage is probably the consequence of the widespread antimicrobial usage among food-producing animals in Europe.

Acknowledgements

This study was partially supported by the Interdisciplinary Centre of Research in Animal Health, from Fundaçao para a Ciência e a Tecnologia, Lisbon, Portugal and by a grant (No. MIP-061/2012) from the Research Council of Lithuania.

References


