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Short communication

First report of swine-associated methicillin-resistant *Staphylococcus aureus* ST398 in Lithuania

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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 SCC*mec* 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.

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Table 1. Molecular characteristics of the four MRSA isolates from pigs.

Isolate	Sample source	Origin	SCC*mec*	ST	*spa*	PFGE pattern[a]	PVL
LT1	Nasal swab	Pig	V	398	t011	A1	neg
LT2	Nasal swab	Pig	V	398	t011	A2	neg
LT3	Nasal swab	Pig	V	398	t011	B	neg
LT4	Nasal swab	Pig	V	398	t011	A1	neg

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

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

Isolate	Resistance patterns	Resistance genes
LT1	OXA, CLI, ERY, Q/D, TET	*tetM*, *tetK*, *ermB*
LT2	OXA, CLI, ERY, Q/D, TET, SXT	*tetM*, *tetK*, *ermB*, *dfrK*
LT3	OXA, CLI, ERY, Q/D, TET	*tetM*, *tetK*, *ermB*
LT4	OXA, CLI, ERY, Q/D, TET	*tetM*, *tetK*, *ermB*

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, SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 methicillin-resistant European sub-lineage is probably the consequence of the widespread antimicrobial usage among food-producing animals in Europe.

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