

Occurrence and characteristic of methicillin-resistant *Staphylococcus aureus* on pig farms in the Czech Republic

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Abstract

In recent years, a negative trend of increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has been noted worldwide. In addition to their prevalence in humans, MRSA strains have also been detected in animals, both pets and food-producing animals, especially in pigs. At the same time, transmission of MRSA strains from animals to humans has been documented. This study brings results from the first nationwide survey on the prevalence of MRSA in herds of breeding pigs, carried out throughout the Czech Republic in accordance with the Commission Decision 2008/55/EC, including brief characteristics of isolates. In 2008, a total of 283 pooled samples (dust swabs from partitions between pens) from the holdings of breeding pigs were analyzed. In five cases, MRSA isolates were detected, with a prevalence of 1.8%. All the isolated strains belonged to a single sequence type ST398. It may be said that at present, the prevalence of MRSA on pig farms does not pose a significant general epidemiological risk for the human population.

MRSA, bacterial zoonoses, sequence type, toxins, antibiotic resistance

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were first described in hospitalised patients in Great Britain (Jevons 1961). These strains rapidly spread worldwide, particularly in the 1980s and 1990s, and their epidemiological importance continues to grow. The important fact is an increasing amount of data on transmission of MRSA between animals and humans. Since 2005, MRSA has been reported in animals (Voss et al. 2005). However, the first cases of detection had been described much earlier (Devriese et al. 1972). At present, these strains, livestock associated MRSA (LA-MRSA), are intensively monitored in veterinary medicine since colonized or infected animals may easily contribute to the spreading of MRSA strains not only to the staff but, in case of food animals, also to raw materials to be processed and consumed (Lee 2003). The LA-MRSA strains were reported in cattle, horses and poultry (Devriese and Hommez 1975; Seguin et al. 1999; Lee 2003) and especially in pigs (Voss et al. 2005).

The first pan-European survey on the occurrence of MRSA in herds of breeding pigs was carried out in the EU member states in accordance with the Commission Decision 2008/55/EC in 2008. Results of the survey in the Czech Republic, including characteristics of the isolates, are presented here.

Materials and Methods

Sample collection on pig farms

Samples of sterile swabs were collected in 2008 on 283 pig farms throughout all regions of the Czech Republic. Holding selection, sample collection and processing were carried out in accordance with methods published

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in the Commission Decision 2008/55/EC. On each holding, 5 samples were collected to make a single pooled sample for laboratory analysis. The samples were collected from dust accumulated on metal partitions separating individual pens.

Sample analysis and methicillin-resistant *Staphylococcus aureus* culture

The method was in accordance with the Commission Decision 2008/55/EC protocol. In presumptive colonies grown on the chromogenic agar, colour and morphology of cultures in Gram-stained preparations were assessed. The colonies indicative of being MRSA were subcultured on blood agar (Oxoid). The MRSA strains were confirmed by a PBP2a latex agglutination test (MRSA-Screen test, Denka Seiken Co., Japan) with simultaneous identification of the *mecA* gene by PCR (Poulsen et al. 2003). The strains were stored in a kryobank (ITEST) at -80 °C for later testing. One MRSA strain (labelled J3420) perished in the course of the study and was lost to further analyses.

Typing of methicillin-resistant *Staphylococcus aureus*

The PCR method was used to study the ability of MRSA strains to produce enterotoxins - detection of the *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei* and *sej* genes (Monday and Bohach 1999; Løvseth et al. 2004), exfoliatins ETA and ETB (Jackson and Iandolo 1986; Lee et al. 1987), PVL (Lina et al. 1999) and TSST-1 (Monday and Bohach 1999). Macrorestriction analysis using pulsed-field gel electrophoresis (PFGE) (Pantucek et al. 1996) was carried out using the *Sma*I restriction enzyme.

Additionally, staphylococcal chromosome cassette *mec* (SCC*mec*) types were studied in these strains (Milheirico et al. 2007), and *spa* typing (Harmsen et al. 2003) and multi locus sequence typing (MLST) (Enright et al. 2000) were performed.

Resistance to antimicrobial agents

The minimum inhibitory concentration (MIC) values for MRSA isolates to 15 antimicrobial agents were determined by the standard broth microdilution method (CLSI 2007) using a commercially available test (Trios) and by the Phoenix automated system (Becton Dickinson, USA). Isolates resistant to erythromycin and susceptible to clindamycin were examined using the disc diffusion test (D-zone test) with erythromycin (15 µg) and clindamycin (2 µg) discs (Oxoid) according to the method of (Fiebelkorn et al. 2003) to detect inducible macrolide, lincosamide and streptogramin B (iMLS_B) resistance.

Results

Occurrence of methicillin-resistant *Staphylococcus aureus* on pig farms

Of the 283 studied samples, MRSA was detected in only five holdings. It means that the prevalence of MRSA in herds of breeding pigs in the Czech Republic was 1.8%. All five isolates of *S. aureus* showed positive reaction to the presence of both the PBP2a protein and the *mecA* gene.

Molecular typing

None of the four MRSA strains available for further molecular characterization carried genes for the production of enterotoxins, exfoliatins, Panton-Valentine leukocidin and TSST-1. Using PFGE, all the isolates were classified as nontypeable (NT). Characteristics of the chromosome cassettes, *spa* and MLST are listed in Table 1.

All isolates showed ST398 and were closely related by *spa* typing. Two isolates shared *spa* type t2346, but they differed by SCC*mec* types.

Resistance to antimicrobial agents

Table 2 shows the minimum inhibitory concentration values for the studied antibiotics. With

Table 1. The molecular characteristics of methicillin-resistant *Staphylococcus aureus* from pig farms

Isolate	SCC <i>mec</i> type	MLST type	Allelic profile								<i>spa</i> type	Tandem repeats
J2692	V	ST 398	3	35	19	2	20	26	39	t034	08-16-02-25-02-25-34-24-25	25
J4160	V	ST 398	3	35	19	2	20	26	39	t2346	08-16-02-25-25-34-24-25	34-24-24-25
J4443	V	ST 398	3	35	19	2	20	26	39	t4659	08-16-02-25-25-24-24-24-25	24-24-24-25
J4491	IV	ST 398	3	35	19	2	20	26	39	t2346	08-16-02-25-25-34-24-24-25	34-24-24-25

SCC*mec* - staphylococcal chromosome cassette, MLST - multi locus sequence typing

Table 2. Minimum inhibitory concentrations of the tested antibiotics

Antimicrobial agent	Strain no. / MIC (mg/l)			
	J2692	J4160	J4443	J4491
Chloramphenicol	16	4	2	2
Tetracycline	> 8	> 8	> 8	> 8
Erythromycin	> 4	> 4	≤ 0.25	> 4
Clindamycin	> 4	> 8	> 4	iMLS _B *
Ciprofloxacin	4	0.5	0.5	0.5
Gentamicin	1	> 8	1	> 8
Teicoplanin	≤ 1	≤ 1	≤ 1	≤ 1
Vancomycin	≤ 1	≤ 1	≤ 1	≤ 1
Tigecycline	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.06
Nitrofurantoin	8	8	8	8
Daptomycin	≤ 1	≤ 1	≤ 1	≤ 1
Co-trimoxazole	1	1	16	1
Fusidic acid	≤ 2	≤ 2	≤ 2	≤ 2
Linezolid	1	1	1	1
Rifampicin	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5

*positive D test, MIC - minimum inhibitory concentration, iMLS_B - inducible macrolide, lincosamide and streptogramin B resistance

the exception of the J4443 strain, the tested isolates were resistant to macrolides. In case of the J4491 strain, inducible clindamycin resistance was detected. Resistance to tetracycline was found in all isolates. All MRSA isolates were susceptible to vancomycin, teicoplanin, tigecycline, daptomycin, fusidic acid, nitrofurantoin, linezolid and rifampicin.

Discussion

LA-MRSA strains were found on pig farms in the Czech Republic. The results show that LA-MRSA has spread in herds of breeding pigs in the Czech Republic, similar to numerous other European countries. However, the prevalence detected

in the Czech Republic is low (1.8%) compared to that in other EU member countries participating in the study. For instance, MRSA prevalence rates in Italy, Belgium, Germany and Spain ranged from 35% to 50%, depending on the farm types. On the other hand, the study found no LA-MRSA on pig farms in the United Kingdom (285 investigated farms), Sweden (202), Lithuania (82) and Ireland (189) (EFSA 2009). Methods described by the Commission Decision 2008/55/EC are concerned with analyzing environmental samples only. However, isolates obtained from dust may not objectively reflect the actual prevalence of MRSA in pigs or breeders and their family members.

The LA-MRSA strains were detected at five Czech farms located in 5 different districts. A close association with e.g. animal (staff) movement between these farms could not be reliably confirmed.

Molecular typing of the strains showed the presence of ST398 on Czech pig farms. Moreover, the *spa* types of Czech porcine MRSA strains are closely related to *spa* types t011 and t108, the most predominant ones among pig-related MRSA strains. Similar results were reported by most countries participating in the EU study (EFSA 2009). High clonality of MRSA strains from pigs was documented and porcine strains of ST398 were found in France, the Netherlands, Germany and Denmark (Armand-Lefevre et al. 2005; de Neeling et al. 2007; Guardabassi et al. 2007; Witte et al. 2007). Although the isolates of a clone represented by ST398 do not seem to be frequent in human MRSA isolates, several cases of colonization and infection in humans in contact with pigs were reported (Armand-Lefevre et al. 2005; Witte et al. 2007; EFSA 2009). MRSA ST398 can occasionally be introduced into hospitals as a result of community-acquired human infections (EFSA 2009). Isolation of methicillin-susceptible *S. aureus* (MSSA) isolates of ST398 belonging to the separate pig-related biotype from 3 bacteraemic cases suggests a high virulence potential of these strains and close surveillance of ST398 human infections is highly advocated (van Belkum et al. 2008). In the Czech Republic, a study of nasal carriage of MRSA strains among veterinarians carried out in 2008 did not show the presence of ST398 among the screened persons (Zemlickova et al. 2009).

The ST398 MRSA sequence type is characterized by the presence of SCC*mec* types IV

and V, absence of genes responsible for production of enterotoxins, exfoliatins A and B, Panton-Valentine leukocidin and TSST (van Duijkeren et al. 2008). The ST398 type strains are not cleaved by restriction endonuclease *Sma*I and according to that characteristic they are nontypeable using PFGE. The presented results confirmed that the tested MRSA isolates did not carry any of the studied genes for enterotoxins, exfoliatins, PVL and TSST-1 toxins.

The tested isolates were resistant to at least four types of antibiotics. In accordance with results of Dutch (de Neeling et al. 2007) study, all isolates were also resistant to tetracycline. Since tetracycline accounts for more than a half of the total amount of antibiotics used in Czech veterinary medicine (personal communication, Alfred Hera), resistance to tetracycline in porcine MRSA may be assumed to be directly associated with its preferential use.

The study presented results of the first official monitoring of LA-MRSA in pig farming in the Czech Republic. Presently, the prevalence of MRSA on pig farms (1.8%) was very low compared to other European countries. All isolated strains belonged to a single sequence type, ST398, and none of the MRSA isolates carried genes for tested toxins. Monitoring of the occurrence of LA-MRSA should be recommended in other commodities (in particular milk and dairy products) directly entering the human food chain, from which *S. aureus* strains are frequently isolated.

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