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Antimicrobial resistance and population structure of *Staphylococcus epidermidis* recovered from pig farms in Belgium

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ABSTRACT

Pigs are known to harbour a variety of staphylococcal bacteria, including *Staphylococcus epidermidis*, in the upper respiratory tract. The aim of the present study was to determine the prevalence, genetic diversity, virulence and antimicrobial resistance of *S. epidermidis* in healthy pigs, as well as to identify the potential role of pigs as a reservoir of zoonotic infection.

The overall prevalence of *S. epidermidis* carriage was 28%, with approximately half of the pigs tested (13.5%) carrying methicillin-resistant *S. epidermidis* (MRSE). Some isolates belonged to multilocus sequence types, associated with healthy human carriers or healthcare personnel (ST88, ST210) whereas others were related to animal or environmental strains (ST100, ST273). Most MRSE isolates carried SCC*mec* type IV, with SCC*mec* type V or a non-typeable SCC*mec* detected in the remaining isolates. Both MRSE and methicillin-susceptible *S. epidermidis* isolates showed a degree of antimicrobial resistance, with most resistant to tetracycline and/or trimethoprim antimicrobial drugs. Isolates subjected to micro-array analysis carried the antimicrobial resistance genes *tet*(K), *tet*(M) and *dfrS1*, while half carried the arginine catabolic element (ACME) associated with colonisation. Some MRSE ST273 strains also carried the *ica* operon involved in biofilm formation. These research findings provide insight into the population structure and characteristics of *S. epidermidis* carried by healthy pigs, suggesting a role for these strains as a potential reservoir for antimicrobial and virulence genes and indicating that exchange of strains might occur between pigs and humans.

Introduction

Porcine tonsils and the nasal cavity can act as a site of colonisation by opportunistic pathogens, including staphylococcal bacteria (Weese et al., 2014). In Belgium, methicillin-resistant *Staphylococcus epidermidis* (MRSE) has been found to be a major constituent of the methicillin-resistant non-*Staphylococcus aureus* staphylococci (MRNAS) flora in pig nostrils (Vanderhaeghen et al., 2012).

S. epidermidis colonises the skin and mucous membranes of mammalian species and is acknowledged as an important opportunistic pathogen in humans (Otto, 2009). Approximately 70–95% of the *S. epidermidis* strains circulating in human hospitals have been estimated to be methicillin-resistant, and most also display resistance to other classes of antimicrobial drugs (Otto, 2009). In staphylococci, methicillin resistance is mediated by acquisition of *mecA* or *mecC* genes, located on the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*) (García-Álvarez et al., 2011). In some *S. epidermidis* strains, SCC*mec* has been found to be associated with the arginine catabolic element (ACME), a genomic island which might contribute to enhanced fitness and an ability to colonise the host (Barbier et al., 2011).

In veterinary medicine, *S. epidermidis* is one of the main aetiological agents of intra-mammary infections in ruminants (Vanderhaeghen et al., 2014) and can also be involved in various types of infectious disease in companion animals (Kern and Perreten, 2013). The aim of the present study was to investigate the prevalence, genetic diversity, virulence and antimicrobial resistance characteristics of *S. epidermidis* in pigs, as well as to determine the role that pigs might play as potential reservoirs of zoonotic *S. epidermidis* for the human population.

Materials and methods

Sampling and bacterial isolation methods

Ten Belgian farms, previously shown to harbour methicillin-resistant *S. aureus* (MRSA) sequence type (ST) 398 positive pigs (Vanderhaeghen et al., 2012), were selected for the study. Four of the farms (referred to as F1, F2, F5, F9) contained only

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fattening pigs, four (F6, F7, F8 and F10) operated on a farrow-to-finisher basis (with sows, piglets and fattening pigs present) and the remainder (F3, F4) were breeding farms (with sows and piglets only).

Nasal swabs were taken from 10 healthy pigs of each representative age group, resulting in a total of 200 samples from 60 sows, 60 piglets and 80 fattening pigs. Bacterial isolation was undertaken as described previously (Vanderhaeghen et al., 2012). Briefly, samples were enriched in 7.5% NaCl Brain Heart Infusion (BHI) broth (Becton Dickinson) at 37 °C overnight. Isolation was performed on ChromID MRSA agar (BioMérieux), Columbia Agar (Oxoid) supplemented with 5% sheep blood (BioMérieux) and 3.5 mg/L cefoxitin (Sigma–Aldrich), ChromID *S. aureus* agar (BioMérieux), and Columbia colistin-aztreonam agar with 5% sheep blood (Oxoid). Colonies were sub-cultured on blood agar plates and visually inspected after 48–72 h at 37 °C. Each colony showing a unique *S. epidermidis* morphotype was included in the study.

Identification, mecA *detection and SCC*mec *typing*

DNA was extracted as previously described (Vanderhaeghen et al., 2012). MRSE and methicillin-susceptible *S. epidermidis* (MSSE) isolates were initially identified by the triplex PCR, developed for detection of the staphylococcal specific 16S rDNA gene, the *S. aureus* specific *nuc1* gene, and the methicillin-resistance *mecA* gene (Maes et al., 2002). Identification at the species level was performed by tDNA intergenic spacer analysis (Supré et al., 2009). Detection of the *mecC* gene and SCC*mec* typing were performed as described previously (Vanderhaeghen et al., 2012, 2013).

Macrorestriction pulse field gel electrophoresis

DNA from each *S. epidermidis* isolate was analysed by SmaI macrorestriction and pulsed field gel electrophoresis (PFGE), using a CHEF Mapper system (Bio-Rad Laboratories). Plugs were prepared according the protocol of Argudín et al. (2010) with some modifications. The cell suspension was adjusted at an optical density (OD) of 1.8 at 610 nm, and a 2 h lysis step at 37 °C with a solution consisting of 6 mM Tris/HCl, 1M NaCl, 100 mM EDTA, 0.5% sodium lauryl sarcosine (SLS), 0.5% Brij58, and 1 mg/mL lysozyme (all from Sigma–Aldrich) was added prior the lysis step undertaken using 0.5 M EDTA, 1% SLS and 1 mg/mL proteinase K.

The electrophoresis conditions were 6 V/cm in 0.5 × TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA; pH 8) at 11.3 °C and runs lasted 23 h with switch times from 5 to 35 s using 1.5% agarose gels. PFGE profiles were compared using BioNumerics software version 6.6 (Applied Maths). A dendrogram was derived from Dice similarity indices, based on the unweighted pair group method with arithmetic averages. According to the criteria established by Miragaia et al. (2008), a cut-off similarity value of 79% was used to establish PFGE types.

Multilocus sequence typing

One to three MRSE isolates, representing each PFGE type grouping of MRSE isolates, were further analysed by multilocus sequence typing (MLST[1]). Amplicons of the genes were purified and sequenced by Macrogen. The *S. epidermidis* MLST website[2] was used to assign STs and clonal complexes (CCs).

Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) of 19 antimicrobial drugs were determined using custom veterinary international Sensititre staphylococci plates (EUST; Trek Diagnostics System) according to the manufacturer's instructions. The interpretation of MIC values was undertaken according to the cut-off values stated by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) for coagulase negative staphylococci (CoNS) and/or *S. aureus*.[3]

For each antimicrobial agent, the isolates were classified as belonging to the wild-type (WT) population or to the non-wild type (NWT) population. Results regarding the MRSE isolates have been published previously (Vanderhaeghen et al., 2012). MRSE (*mecA*-positive isolates) classified as cefoxitin-susceptible were also tested by the disk diffusion method using oxacillin discs (Oxoid) according to the Clinical and Laboratory Standards Institute (2012).

DNA microarray-based typing and detection of the ica operon

Microarray analysis was performed for selected isolates on the basis of the PFGE dendrogram using the Identibac *S. aureus* Genotyping DNA Microarray (Alere Technologies) according to the manufacturer's instructions. Additionally, all isolates were investigated by PCR for the presence of the *ica* operon involved in biofilm production as previously described (Arciola et al., 2005).

[1] See: http://www.mlst.net/.
[2] See: http://sepidermidis.mlst.net/.
[3] See: http://www.eucast.org/mic_distributions/.

Statistical methods

To assess the discriminatory power of the PFGE method, the discrimination indices and the 95% confidence interval (CI) was calculated using Simpson's index of diversity (SID) as previously described (Hunter and Gaston, 1988; Grundmann et al., 2001). Statistical comparison of the prevalence of antimicrobial resistance was performed by the chi-square test.

Results

S. epidermidis *prevalence in study animals*

A total of 70 *S. epidermidis* isolates were recovered from 56 pigs (28%, 95% CI: 21.9–35%) on nine farms (90%, 95% CI: 55.5–100%; Table 1). The *mecA* gene was detected in 28 isolates from 27 pigs (13.5%, 95% CI: 9.1–19%) on five farms (50%, 95% CI: 18.7–81%). Twelve pigs (four fattening pigs, four sows and four piglets) carried more than one isolate; six were positive for both MRSE and MSSE isolates.

*SCC*mec *typing and population structure*

Among the 28 MRSE isolates, four different SCC*mec* cassettes were detected: IVa (2B) ($n = 20$ isolates), IVc (2B) ($n = 5$ isolates), V (5C2) ($n = 2$ isolates) or a single non-typeable isolate, only positive for *mecA* and the *ccr* complex (Vanderhaeghen et al., 2012). The 70 isolates yielded 47 SmaI-PFGE patterns (SID: 0.984, 95% CI: 0.974–0.994), which were grouped into 18 PFGE types, named from A to R (SID: 0.915, 95% CI: 0.890–0.940; Fig. 1).

One major clone was detected at a Dice similarity index of 73.8%, grouping 40% of all isolates. It clustered PFGE types A–E and included both MSSE and MRSE. The latter were classified as ST100 and carried SCC*mec* cassettes IVc, V or non-typeable. The isolates of this clone were recovered from animals of different age groups, with 82% being from sows and piglets (Fig. 1). ST100 positive strains and isolates with related PFGE types were found in animals belonging to eight farms, including farms housing fattening pigs only (F1, F2, F5), breeding stock (F3, F4) and farrow-to-finishers (F6, F8 and F10).

Table 1
Prevalence of *S. epidermidis* in study pigs.

Farm identity	Pig type	Number of animals sampled	Number of positive animals	Number of isolates	Number of MRSE	Number of MSSE
F1	FP	10	1	1	1	0
F2	FP	10	6	10	6	4
F3	S	10	3	3	0	3
	P	10	7	8	1	7
F4	S	10	3	4	2	2
	P	10	6	6	6	0
F5	FP	10	3	4	0	4
F6	S	10	0	0	0	0
	P	10	6	6	6	0
	FP	10	6	8	6	2
F7	S	10	0	0	0	0
	P	10	0	0	0	0
	FP	10	0	0	0	0
F8	S	10	0	0	0	0
	P	10	5	6	0	6
	FP	10	3	5	0	5
F9	FP	10	2	2	0	0
F10	S	10	0	0	0	0
	P	10	5	7	0	7
	FP	10	0	0	0	0
Total	S	60	6	7	2	5
	P	60	26	33	13	20
	FP	80	21	30	13	15
	Total	200	53	70	28	40

MRSE, methicillin-resistant *S. epidermidis*; MSSE, methicillin-susceptible *S. epidermidis*; F, farm number; FP, fattening pig; P, piglet; S, sow.