Molecular characterization of spa type t127, sequence type 1 methicillin-resistant Staphylococcus aureus from pigs

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Objectives: The aim of this study was to provide molecular characterization of methicillin-resistant Staphylococcus aureus (MRSA) spa type t127, sequence type (ST) 1 isolates, detected in a European baseline survey in holdings of breeding pigs, to determine phenotypic and genotypic drug resistance and to compare the results with those obtained from a collection of t127, ST1 MRSA and methicillin-susceptible S. aureus (MSSA) clinical isolates.

Methods: Twenty-four t127, ST1 MRSA from dust sampled in different breeding holdings in Italy, Spain and Cyprus were studied, along with 2 t127, ST1 MRSA from fattening pigs and 11 human t127, ST1 MRSA and MSSA. Genotyping was performed using multilocus sequence typing (MLST), spa typing and PFGE. SCCmec elements were characterized by multiplex-PCR and resistance and pathogenicity genes by PCR and microarray.

Results: PFGE patterns separated a porcine cluster (PC) from a human cluster (HC), with 75% similarity. The PC carried SCCmec cassette type V, while all isolates of the HC carried SCCmec cassette type IVa. Kanamycin resistance mediated by aadD, fluoroquinolone and erm(A)-mediated macrolide resistance and the absence of the sakA gene were features of the PC only. All isolates of both clusters were positive for LukE-LukD and LuF-LukS-HlgA leukotoxin genes and one human MSSA harboured Panton–Valentine leucocidin genes.

Conclusions: Despite differences in the host-specific genetic features, the possibility of PC transmission to humans cannot be excluded. MRSA spa type t127, ST1 from pigs possesses several virulence and resistance genes towards major classes of antimicrobials and may represent a serious therapeutic challenge in case of invasive infections in humans.

Keywords: genotyping, molecular epidemiology, MRSA, S. aureus, pigs, zoonoses

Introduction

In recent years pigs and other food animals have been proven to be a source of methicillin-resistant Staphylococcus aureus (MRSA) sequence type (ST) 398 for human infections in many European countries. Conversely, the presence of MRSA spa type t127, ST1 has been only recently reported in pigs and may represent an additional MRSA lineage for infections in humans.1 ST1 belongs to clonal complex (CC) 1, which includes Panton–Valentine leucocidin (PVL)-positive community-associated MRSA (CA-MRSA) also known as USA4002 and PVL-negative ST1 t127, SCCmec IVa MRSA, which are among the most common CA-MRSA in the UK.1 The aim of the study was to provide molecular characterization of t127, ST1 MRSA isolates detected in a European harmonized baseline survey in breeding holdings of pigs, to determine phenotypic and genotypic drug resistance and to explore their genetic relatedness to a collection of clinical MRSA and methicillin-susceptible S. aureus (MSSA) available in Italy and Denmark.

Materials and methods

Thirty-seven t127, ST1 S. aureus isolates were included in the study. Twenty-four MRSA isolates originated from dust samples and were collected in holdings of breeding and production pigs screened during the European Community baseline survey (Commission Decision 2008/55/EC), of which 21 from Italian, 2 from Spanish and 1 from Cypriot holdings were included. Two more isolates, randomly selected among the 7 spa type t127, ST1 MRSA obtained from nasal swabs in finishing pigs surveyed in Italy in 20082 and 11 S. aureus human clinical isolates
4 MSSA and 7 MRSA, from Italy and Denmark) with unknown epidemiological relationships with pig farming were also included for comparison purposes (see Figure 1). Susceptibility testing was performed in 96-well microtitre plates (Trek Diagnostic Systems, Westlake, OH, USA) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org) epidemiological cut-offs. The following drugs were tested: ampicillin, oxacillin, ciprofloxacin, clarithromycin, clindamycin, erythromycin, gentamicin, kanamycin, linezolid, tetracycline, tiamulin, trimethoprim and vancomycin.

MRSA and MSSA isolates were genotyped as described in a recent paper, by DNA sequencing of the X region of S. aureus protein A (spa typing), with repeats and spa types determined by Ridom StaphType software (Ridom GmbH, Würzburg, Germany) and by performing multilocus sequence typing (MLST). Similarly, typing of the staphylococcal cassette chromosome mec (SCCmec) and subtyping were performed using multiplex PCR. PFGE was performed as described previously. Smal-digested DNA PFGE patterns were analysed using BioNumerics 4.61 software (Applied Math, St-Martens-Latem, Belgium).

A selection of 30 isolates clustering in all different PFGE patterns were further characterized by means of a previously described DNA microarray (ArrayTubes, Alere GmbH, formerly known as Clondiag GmbH) for the detection of pathogenicity, antimicrobial resistance genes and strain-specific markers of S. aureus, including the accessory gene regulator (agr) gene cluster and superantigen-toxin encoding genes. All these tests were performed and interpreted according to manufacturer recommendations. All these isolates were further tested by PCR for the detection of the mecA gene, haemolysin genes, leucocidins, exfoliative toxins and tst genes, as described in a recent paper, and for tet(K), tet(L), tet(M) and tet(O) genes.

**Results**

The Smal PFGE results (Figure 1) showed that the isolates under study represented 18 different PFGE profiles, separated by the analysis into two major clusters; a porcine cluster (PC) consisting of PFGE profiles A–I (n = 25 isolates) and a human cluster (HC; n = 12 isolates) consisting of PFGE profiles J–R, with >75% similarity, while 7 out of 9 profiles within the PC showed a minimum similarity of 90%. The only MRSA detected in one Cypriot breeding holding grouped within the HC. All isolates of the PC harboured the mecA gene in the type V SCCmec cassette, while all MRSA isolates of the HC were SCCmec type IVa. A 100% concordance was found between micro-array and PCR test results for the mecA and the virulence factors studied. The determination of the quorum sensing system agr groups showed that all isolates belong to the agr type III group. All isolates were positive for the genes encoding the components of LukE-LukD and LuF-LukS-HlgA leukotoxin families, and for the lukV-var1 genes, and were all negative for lukM. All isolates, but one human MSSA from Italy, were PVL negative. All isolates carried the haemolysins hla, hlb, hld and hiII. All porcine isolates were positive for the hib gene, absent in 11/12 isolates of the HC, that conversely were found to be positive for the sakA gene, as shown in Table 1. None of the isolates was positive for the exotoxin genes (eta, etb, etd or tst). Protease genes spaI and spaB were present in all isolates. The results for other virulence genes, the enterotoxin genes and the phenotypes and genotypes of resistance are shown in Table 1. None of the isolates studied harboured the mrs(A) gene or the streptogramin A acetyltransferase genes vat(A) and vat(B). All isolates were susceptible to linezolid and vancomycin and did not carry any of the genes involved in their resistance mechanisms.

**Discussion**

The genetic characterization of spa type t127, ST1 S. aureus (MRSA or MSSA) isolates allows the distinction of a PC from a HC. One exception to the separation of the isolates of pig and human origin into a PC or HC was the only MRSA isolate detected in Cypriot pig holdings, showing a PFGE profile and most of the genotypic features in common with the HC. One possible explanation for this discrepancy is that the isolate was introduced into that pig holding environment by a human carrier. Additionally, the two Italian isolates from pig finishing holdings examined are indistinguishable by PFGE from other isolates originating from breeding holdings; this suggests their transmission along the pig production chain. Among isolates included in this study, some genetic features were found that correlate with the PFGE attribution of isolates to one of the two clusters.

The absence of the staphylokinase gene sakA, encoding one of the plasminogen activators, in all isolates of the PC had already been noticed in S. aureus from cattle, so that its presence may be considered the result of an adaptive advantage for the colonization of human hosts only.

The single PVL-positive isolate, a human MSSA from Italy, demonstrates that spa type t127, ST1 isolates are capable of acquiring such virulence factors besides other members of the leukotoxin family like LukE-LukD and LuF-LukS-HlgA.

The antimicrobial resistance gene pattern observed in the two clusters is different and is likely to be the result of different selection pressures in the community and in the pig production system. blaZ-mediated β-lactam resistance was a feature common to both clusters, while mecA-mediated β-lactam resistance was observed in the type V SCCmec cassette in a characteristic observed in the PC only, with all isolates of the HC carrying SCCmec type IVa.

Kanamycin resistance, when not associated with gentamicin resistance and the presence of the bifunctional gene aacA-aphD, is mediated by aadA genes only in the PC and by aphA genes only in the HC. A tetracycline, macrolide-lincosamide (ML) and fluoroquinolone resistance pattern seems a specific feature of isolates of the PC. Tetracycline resistance appears to be a constant finding of porcine MRSA of all STs in the European Union. Similarly the high frequency (15/18, 83%) of one or more genes (especially erm) involved in ML resistance among the PC could be related to the long-term use of macrolides as growth promoters and as therapeutic agents in pigs. Since lincosamide resistance was observed in most (13/14) of the macrolide-resistant isolates, this suggests constitutive expression of the erm genes detected. The presence of the erm(A) gene (alone or in combination with the erm(C) gene) and the vga(A) putative ABC transporter gene, the latter conferring combined resistance to streptogramin A antibiotics, lincosamides and pleuromutilins, seems another genetic feature of the PC, while the presence of the streptogramin B hydrolase gene vgb(A) appears to be a feature specific to the HC. An exception is one pig isolate from Spain (VE08-01769).
An elevated clindamycin MIC of 1 mg/L in the absence of macrolide resistance observed in four isolates of the PC was attributed to either the \textit{lnu(A)} gene encoding a lincosamide nucleotidyltransferase or to the aforementioned \textit{vga(A)} gene, similar to what has been observed in porcine ST398 MRSA, with all \textit{vga(A)}-positive isolates also showing the pleuromutilin resistance phenotype (tiamulin MICs > 4 mg/L). All \textit{sat}-positive isolates of the HC also harboured the aminoglycoside resistance gene \textit{aphA}, which is suggestive of a linkage of the two genes and of the presence of Tn5405-like elements. Of interest is the simultaneous presence of these two genes and the macrolide resistance gene \textit{erm(C)}, a feature unique to this cluster (see Table 1).

In conclusion, the characterization of t127, ST1 MRSA from pigs provides evidence that this lineage possesses several genes encoding virulence factors, superantigenic toxins and antimicrobial resistance besides those involved in \textit{mecA}-mediated \(\beta\)-lactam resistance. This study also confirms that t127, ST1 \textit{S. aureus} is capable of acquiring PVL-toxin genes, detected in one human MSSA isolate. Although there are differences in the host-specific genetic features between the two clusters, the possibility of transmission of the porcine MRSA t127, ST1 to humans cannot be excluded, and may represent a serious therapeutic challenge in case of invasive infections.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\% similarity & PFGE Smal & Strain & Country & Origin & MRSA/MSSA Group \\
\hline
22610 & Italy & Breeding pigs & MRSA & A \\
30616 & Italy & Breeding pigs & MRSA & A \\
31695 & Italy & Breeding pigs & MRSA & A \\
19490 & Italy & Breeding pigs & MRSA & B \\
19722 & Italy & Breeding pigs & MRSA & B \\
21086 & Italy & Breeding pigs & MRSA & B \\
21086 & Italy & Breeding pigs & MRSA & B \\
21554 & Italy & Breeding pigs & MRSA & B \\
21554 & Italy & Breeding pigs & MRSA & B \\
21746 & Italy & Breeding pigs & MRSA & B \\
21746 & Italy & Breeding pigs & MRSA & B \\
23701-5-10 & Italy & Breeding pigs & MRSA & B \\
23701-5-10 & Italy & Breeding pigs & MRSA & B \\
27506-9 & Italy & Breeding pigs & MRSA & B \\
27506-9 & Italy & Breeding pigs & MRSA & B \\
28814-6 & Italy & Breeding pigs & MRSA & B \\
28814-6 & Italy & Breeding pigs & MRSA & B \\
5665-6 & Italy & Breeding pigs & MRSA & B \\
5665-6 & Italy & Breeding pigs & MRSA & B \\
17910-1 & Italy & Breeding pigs & MRSA & C \\
17910-1 & Italy & Breeding pigs & MRSA & C \\
17931-6 & Italy & Breeding pigs & MRSA & C \\
17931-6 & Italy & Breeding pigs & MRSA & C \\
22845-1 & Italy & Breeding pigs & MRSA & C \\
22845-1 & Italy & Breeding pigs & MRSA & C \\
23206 & Italy & Breeding pigs & MRSA & C \\
23206 & Italy & Breeding pigs & MRSA & C \\
25729 & Italy & Breeding pigs & MRSA & C \\
25729 & Italy & Breeding pigs & MRSA & C \\
28816 & Italy & Breeding pigs & MRSA & C \\
28816 & Italy & Breeding pigs & MRSA & C \\
VE08-01531 & Spain & Breeding pigs & MRSA & C \\
VE08-01531 & Spain & Breeding pigs & MRSA & C \\
17911 & Italy & Breeding pigs & MRSA & D \\
17911 & Italy & Breeding pigs & MRSA & D \\
27080 & Italy & Breeding pigs & MRSA & E \\
27080 & Italy & Breeding pigs & MRSA & E \\
13049 & Italy & Breeding pigs & MRSA & F \\
13049 & Italy & Breeding pigs & MRSA & F \\
23122 & Italy & Breeding pigs & MRSA & G \\
23122 & Italy & Breeding pigs & MRSA & G \\
VE08-01769 & Spain & Breeding pigs & MRSA & H \\
VE08-01769 & Spain & Breeding pigs & MRSA & H \\
27388 & Italy & Breeding pigs & MRSA & I \\
27388 & Italy & Breeding pigs & MRSA & I \\
106 & Italy & Human & MSSA & J \\
106 & Italy & Human & MSSA & J \\
537 & Italy & Human & MSSA & K \\
537 & Italy & Human & MSSA & K \\
696 & Italy & Human & MSSA & L \\
696 & Italy & Human & MSSA & L \\
843 & Denmark & Human & MRSA & M \\
843 & Denmark & Human & MRSA & M \\
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868 & Italy & Human & MSSA & N \\
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226 & Italy & Human & MRSA & O \\
45552 & Denmark & Human & MRSA & O \\
45552 & Denmark & Human & MRSA & O \\
COD82 & Italy & Human & MRSA & O \\
COD82 & Italy & Human & MRSA & O \\
COD83 & Italy & Human & MRSA & O \\
COD83 & Italy & Human & MRSA & O \\
53054 & Denmark & Human & MRSA & P \\
53054 & Denmark & Human & MRSA & P \\
3194-3 & Cyprus & Breeding pigs & MRSA & Q \\
3194-3 & Cyprus & Breeding pigs & MRSA & Q \\
40445 & Denmark & Human & MRSA & R \\
40445 & Denmark & Human & MRSA & R \\
\hline
\end{tabular}
\caption{SmaI PFGE results of 37 spa type t127, ST1 \textit{S. aureus} isolates (33 MRSA and 4 MSSA) from pigs and humans.}
\end{table}
Table 1. Selected virulence genes, enterotoxin genes, antimicrobial resistance phenotypes and antimicrobial resistance genotypes of the spa type t127, ST1 S. aureus (MRSA and MSSA) isolates studied

<table>
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<th>ID #</th>
<th>hib</th>
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<th>lukX</th>
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BLA, β-lactams; AMP, ampicillin; OXA, oxacillin; TET, tetracycline; KAN, kanamycin; GEN, gentamicin; STH, streptothricin; TMP, trimethoprim; CIP, ciprofloxacin; TIA, tiamulin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; VGA, virginiamycin A; VGB, virginiamycin B.

aNo tet(K), tet(M), tet(L) or tet(O) genes detected.
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Transparency declarations
None to declare.

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