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Short communication

Antimicrobial resistance and population structure of *Staphylococcus aureus* recovered from pigs farms

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ABSTRACT

Staphylococcus aureus is a burden in human and veterinary medicine. During the last decade, an increasing number of studies reported the presence of livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) clonal complex (CC) 398 in pigs. During 2013, a survey was performed in pig farms ($n = 328$) randomly selected over Belgium, to monitor the current epidemiological situation of LA-MRSA among asymptomatic pigs and compare with former data to determine possible evolutions. Per farm, nose swabs were taken from 20 animals and pooled. MRSA was detected in 215 farms. Most isolates belonged to CC398 ($n = 211$), and the remaining were ST9/t337 ($n = 1$), ST80/t044 ($n = 2$) and ST239/t4150 ($n = 1$). A large diversity ($n = 19$) of *spa*-types was found in the CC398 isolates. More than 90% of the isolates were non-wild type (NWT) to tetracycline and trimethoprim. NWT isolates were also found for ciprofloxacin (61.1%), clindamycin (64.4%), erythromycin (57.8%), kanamycin (43.1%) and gentamicin (45.5%). Microarray analysis showed that most CC398 isolates carried genes encoding resistance to tetracycline [*tet*(M)], macrolide–lincosamide–streptogramin group [*erm*(B), *erm*(C), *lnu*(A), *vga*(A)], aminoglycosides (*aacA-aphD,aa dD, aphA3, sat*) and/or phenicols (*fexA*). One CC398 isolate carried the multi-resistance gene *cfr*. The non-CC398 isolates carried virulence genes, as the *egc*-like cluster. The ST80 strain carried the Panton–Valentine leukocidin gene and corresponded to the community-acquired (CA-)MRSA ST80-IV European clone. The MRSA prevalence among pigs in Belgium remains similar to previous studies but a larger diversity in *spa*-types has been detected in this study. The recovery of CA-MRSA from livestock indicates that one should remain vigilant to the evolution of LA-MRSA in pigs.

1. Introduction

Staphylococcus aureus is a common facultative pathogenic bacterium, which is associated with a wide spectrum of diseases in both humans and animals (Crombé et al., 2013). It is well known that specific clones of this bacterium harbour multiple resistances to antimicrobial agents which may lead to complications in the treatment of its infections (Lowy, 2003). One of these antimicrobial resistances is resistance to β-lactamase stable β-lactam antimicrobials in methicillin-resistant *S. aureus* (MRSA). MRSA is an important cause of hospital and community acquired infections worldwide, but is not confined to healthcare settings, as it is a growing problem in veterinary medicine (Crombé et al., 2013). In livestock, MRSA was first reported in a case of bovine mastitis (Devriese et al., 1972). But these cases were, as well as most MRSA infections in animals, of human origin until 2005, when a high prevalence of a specific clone of MRSA [clonal complex (CC) 398] was reported in pigs in the Netherlands (Crombé et al., 2013). This clone was later named livestock associated MRSA (LA-MRSA), and it has been found in many animal species all over the world, but most studies have focused in studying its prevalence in pigs (Crombé et al., 2013). In Asia however, MRSA CC9 appears as the most prevalent clone associated with pig farming (Crombé et al., 2013). MRSA strains from the human lineages ST5, ST8, ST22, ST30, and ST45 have also been reported in pigs from Europe, USA, and Africa (Crombé et al., 2013). Since 2007, a baseline study has been performed in Belgium to determine the prevalence of MRSA in poultry, bovines and pigs (Anonymous, 2009a; Crombé et al., 2012; Nemeghaire et al., 2013, 2014). This study corresponded to the active survey that was performed in 2013 in different pig farms randomly selected over Belgium, with the aim of monitoring the current epidemiology of LA-MRSA among asymptomatic pigs.

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2. Materials and methods

2.1. Sampling and isolation methods

Three-hundred and twenty-eight farms were examined during the national survey on pig MRSA in Belgium in 2013. Per farm, nose swabs were taken from 20 animals and pooled. Sampling was performed by the Belgian Federal Agency for the Safety of the Food Chain. Ethical approval was not required for this study under Belgian regulations, as taking a nasal swab does not cause pain, distress or lasting harm. MRSA was isolated using the standard method proposed by EFSA (Anonymous, 2009b) briefly described in Nemeghaire et al. (2013). One *S. aureus* isolate per farm was further analysed.

2.2. DNA extraction, MRSA identification and characterization

DNA was extracted as previously described (Nemeghaire et al., 2013). MRSA identification, *mecA* gene detection, *spa* typing and the *sau1-hsdS1* CC398 PCR reaction was performed as previously described (Nemeghaire et al., 2013). MRSA isolates that were negative in the CC398 PCR were subjected to multi-locus sequence typing (MLST) (http://saureus.mlst.net). The *spa* types were determined with Ridom StaphType software (www.ridom.de/staphtype/) and analysed by the Based Upon Repeat Pattern (BURP) algorithm. *Spa* types were assigned to the same CC if the cost was less than or equal to six (Mellmann et al., 2007). Staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined by the means of two multiplex PCRs (M-PCRs) designed for the detection of the *mec*-complex and *ccr*-complex (Kondo et al., 2007). Subtyping of the SCC*mec* IV was performed as previously described (Kondo et al., 2007).

2.3. Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) of 19 antimicrobials (penicillin, cefoxitin, kanamycin, streptomycin, gentamicin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, tiamulin, chloramphenicol, rifampicin, ciprofloxacin, fusidic acid, tetracycline, trimethoprim, sulfamethoxazole, vancomycin, and mupirocin) were determined using custom veterinary international Sensititre staphylococci plates EUST (Trek Diagnostics System, United Kingdom) according to the manufacturer's instructions. The interpretation of MIC values was according to the epidemiological cut-off values (ECOFFs) of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) for *S. aureus*. Data from the EUCAST MIC distribution website was last accessed 1st June 2015 (http://www.eucast.org). For each antimicrobial agent, the isolates were classified as belonging to the wild-type (WT) or to the non-wild type (NWT) population (Schwarz et al., 2010).

2.4. DNA microarray-based typing and detection of resistance and virulence genes

Thirty-three selected isolates belonging to different CCs and representative of the *spa* types were subjected to microarray analysis. Microarray analysis was performed by the Identibac *S. aureus* Genotyping DNA Microarray (Alere Technologies GmbH, Germany) according to the manufacturer's instructions. A full list including primer and probe sequences is available online (http://alere-technologies.com/).

2.5. Statistical analysis

The number of resistant strains was counted and resistance percentages were calculated. Exact confidence intervals for the binomial distribution were calculated using a visual basic application in Excel. A 95% symmetrical two-sided confidence interval was used with $p = 0.05$.

3. Results

3.1. Prevalence, population structure and SCCmec typing

MRSA was detected in 215 farms [65.6% (95% CI: 61.0–70.0%)] out of 328 farms sampled. Most isolates ($n = 205$) were positive for the *sau1-hsdS1*CC398 PCR. The remaining eight isolates were ST9 (one isolate), ST80 (two isolates), ST239 (one isolate) or ST398 (six isolates) as demonstrated by MLST. A total of 22 different *spa* types were identified (Table 1). The *spa* types t044, t337 and t4150 were found in the CC80, CC9 and CC8 isolates, respectively. Nineteen *spa* types were found among the 211 CC398 isolates, but most were t011 ($n = 180$). SCC*mec* typing showed that the ST9 and ST80 isolates carried SCC*mec* cassettes types V or IVc, respectively (Table 1). The ST239 carried a non-typeable cassette. Most CC398 isolates carried SCC*mec* V (169 isolates), and less carried SCC*mec* IVa (eight isolates), III (two isolates) or IV variant (2BC&5) (one isolate).

3.2. Antimicrobial resistance

All isolates belonged to the WT population for vancomycin (Table 2). Antimicrobial susceptibility was tested on 211 strains out of 215 isolates. The four isolates not tested (one CC8/ST239 and three CC398 isolates) were lost after sampling. As expected, all strains tested belonged to the NWT population for penicillin and cefoxitin. More than 90% of the isolates were NWT for tetracycline (98.6%) and trimethoprim (96.2%). All CC398 strains except one, were tetracycline resistant. A high prevalence of isolates belonging

Table 1
Total number of MRSA isolates corresponding to the different genotypes recovered.

CC/ST (*N*)[a]	*spa* type (*N*)	SCC*mec* [*N*][b]
CC8/ST239 (1)	t4150 (1)	NT [1]
CC9/ST9 (1)	t337 (1)	V (5C2) [1]
CC80/ST80 (2)	t044 (2)	IVc (2B) [2]
CC398 (211)	t011 (180)	III (3A) [2], IVa (2B) [35], V (5C2) [143]
	t034 (6)	V (5C2) [6]
	t1344 (1)	V (5C2) [1]
	t1451 (2)	V (5C2) [2]
	t1456 (3)	IVa (2B) [2], V (5C2) [1]
	t1580 (3)	V (5C2) [3]
	t1985 (2)	V (5C2) [2]
	t2123 (1)	IVa (2B) [1]
	t2370 (2)	V (5C2) [2]
	t2922 (1)	IV (2BC&5) [1]
	t3171 (1)	IVa (2B) [1]
	t3423 (2)	V (5C2) [2]
	t3854 (1)	V (5C2) [1]
	t4432 (1)	V (5C2) [1]
	t4872 (1)	V (5C2) [1]
	t5051 (1)	V (5C2) [1]
	t5452 (1)	V (5C2) [1]
	t6228 (1)	V (5C2) [1]
	t8100 (1)	V (5C2) [1]

N—number of isolates; NT—non-typeable; ST—sequence type.

[a] The clonal complex (CC) assignment was performed by using the *sau1-hsdS1* CC398 PCR reaction (Stegger et al., 2011) and/or the MLST typing. Six CC398 isolates were negative for the *sau1-hsdS1* CC398 PCR, but they were ST398 on the basis of the MLST typing.

[b] The type of SCC*mec* was determined by the combination of *ccr* complex type and *mec* complex class (IWG-SCC, 2009). As suggested by the International Working Group on the Classification of Staphylococcal Cassette Chromosome elements (IWG-SCC, 2009), cassettes showing a combination of *ccr* complexes were considered as variants of recognized SCC*mec* types. Cassettes with more than one *mec-ccr* combination possible, where no *ccr* complex or *mec* complex could be detected were considered as non-typeable (NT).