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Short communication

First report of *lukM*-positive livestock-associated methicillin-resistant *Staphylococcus aureus* CC30 from fattening pigs in Northern Ireland

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ABSTRACT

The increasing number of reports of livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) world-wide attests to the public health concern surrounding this pathogen in animal husbandry and in-contact humans. In Europe, LA-MRSA CC398 is predominant and generally regarded as being of low virulence for animals. Herein we report the recovery of a lineage of LA-MRSA, belonging to CC30, from three pigs in Northern Ireland and which encodes a marker of virulence (*lukM and lukF-P83*) restricted to animal-associated clones of *S. aureus*.

1. Introduction

Over the last decade, LA-MRSA have emerged in the agricultural setting and are considered a public health concern worldwide (Price et al., 2012). Two lineages of particular note have been identified: CC398 being dominant in mainland Europe and CC9 in Asia (EFSA, 2009; Dhup et al., 2015). These clones have been reported in diverse livestock hosts such as pigs, veal calves and poultry where they are mainly associated with asymptomatic colonisation, although disease has been reported sporadically (Verkade and Kluytmans, 2014). Epidemiological studies have shown that LA-MRSA CC398 does not readily transmit between humans, but reports of infections occurring in people having direct contact with livestock (including farm workers, abattoir workers and veterinarians) are increasing (Verkade and Kluytmans, 2014).

Although LA-MRSA was not found in pig holdings in the UK in a European survey conducted in 2008 (EFSA, 2009), LA-MRSA CC398 has subsequently been reported sporadically in horses (Loeffler et al., 2009), bulk tank milk (Paterson et al., 2012), pigs (Hartley et al., 2014; Hall et al., 2015), turkeys (GOV.UK, 2013) and retail pork (Hadjirin et al., 2015); in addition, CC9 has been recovered from retail chicken meat (Dhup et al., 2015). Herein we report the first isolation of a novel LA-MRSA CC30 strain encoding a recognised marker of virulence (*lukM*) from fattening pigs in Northern Ireland identified as a result of passive surveillance.

2. Materials and methods

2.1. Clinical case description and post-mortem investigation

In February 2015, three pigs with signs of ill-thrift (low rate of growth) from a farm in Northern Ireland (NI) were submitted to the Agri-Food and Biosciences Institute Veterinary Sciences Division (AFBI-VSD) for post-mortem investigation. The tentative diagnosis was porcine circovirus type 2 (PCV2). At necropsy, the gross post-mortem findings were unremarkable apart from the pigs being thin. Tissue samples from liver, lung, spleen, joint hocks, brain skin and intestinal contents were screened by immunofluorescence for a panel of common swine viruses including porcine circovirus type 2 (PCV2). Histopathological examination of tissues was not performed due to the poor post-mortem macroscopic findings.

2.2. Microbiological investigations

To investigate for possible bacterial pathogens, a small portion of each tissue sample (approx 10 μl loop) was flamed to remove surface contamination, homogenised for 1 min in 10 ml of

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phosphate buffered saline (PBS) and 10 μl inoculated onto blood agar. Following overnight incubation at 37 °C, varying levels of pure growth of presumptive *Staphylococcus aureus* was obtained from various sites in the three animals. To ascertain whether they were MRSA, colonies from each site were sub-cultured onto MRSA 2 *Brilliance*™ MRSA agar (Oxoid, UK). The cultures were identified using the Biolog ID system (Techno-Path Ltd., UK) and screened for *mecA*, *mecC*, *nuc* and *lukS-PV* by multiplex PCR as described previously (Pichon et al., 2012).

2.3. Phenotypic and genomic characterisation of S. aureus from pigs

For all nine *S. aureus*, the minimum inhibition concentrations (MICs) of antibiotics were determined by agar dilution (Andrews, 2001) and the results interpreted in accordance with EUCAST criteria. The antimicrobials tested were: penicillin, tetracycline, fusidic acid, ciprofloxacin, erythromycin, clindamycin, gentamicin, rifampicin, teicoplanin, vancomycin, daptomycin, linezolid and mupirocin. In addition, susceptibility to cefoxitin was determined by Etest (bioMérieux, Marcy l'Etoile, France). All *S. aureus* were characterised by *spa* typing (Holmes et al., 2005) and subjected to whole genome sequencing (WGS) on Illumina HiSeq Instrument. Sequences accession: EBI project no PRJEB11734. Reads were mapped with bowtie2 against reference sequences for (i) MLST loci (ii) virulence factors (*scn*, *chp*, *sak*, *sea-e*, *seh-j*, *sep*, *ser*, *seu*, *tst*, *eta*, *etb*, *etd*, *lukS/lukF-PV* and *lukM/lukF-P83*) (iii) heavy metal resistance (*czrC*) and (iv) acquired antimicrobial resistance as described previously (Doumith et al., 2015). Genes were considered present when the alignments covered 100% of the reference sequence and showed ≥90% homology with it. SCC*mec* types were deduced based on the detection of the *mec* complex and *ccr* genes by BLAST on assembled genomes generated using Velvet. The genetic relatedness of the isolates was assessed by Single Nucleotide Polymorphism (SNP) analysis. Sequence reads were mapped to the ST30 reference strain 55-2053 (NC_022113) using BWA(0.7.5). SNPs were called using GATK2.6.5. Genetic relatedness was determined using only high quality SNPs (AD genotype = 0.9). Coverage was above 95% of the reference genome. Indels were not considered. SNPs were concatenated and aligned (Ns excluded) for phylogenetic analyses using the Maximum Likelihood method (MEGA 6).

3. Results

Tissues from all three pigs tested negative for PCV2 by immunofluorescence and all other viruses screened for. However, bacterial isolates from a total of nine different tissues from the three pigs were confirmed as *S. aureus*; eight of which identified as *mecA* positive MRSA (Table 1).

All nine *S. aureus* isolates were resistant to penicillin and tetracycline (MICs ≥0.5 and >8 μg/ml, respectively). Moreover, the eight *mecA* positive isolates showed low level resistance to cefoxitin (MIC 8 μg/ml) and the remaining isolate was susceptible (MIC 2 μg/mL) by Etest.

Genetic analysis of these isolates showed they identified as ST30, *spa* type t1749 and encoded the tetracycline resistance marker *tet*(K); all isolates except that recovered from the lung of pig B were *mecA*- and *czrC*-positive and harboured SCC*mec*Vt (Table 1). In addition, all nine isolates encoded the *lukM* and lukF-P83 genes, a marker of virulence restricted to animal lineages (Schlotter et al., 2012; Simpson et al., 2013); *tst* and *lukSF-PV* were not detected but the enterotoxin gene cluster was present. Further, they lacked the genes associated with the immune evasion cluster (IEC) associated with ΦSa3, a recognised marker of human adaptation (Price et al., 2012). SNP-based analysis clustered the pig isolates into the same clade, distant by more than 627 SNPs

Table 1
Genotypic and phenotypic characteristics of *S. aureus* strains isolated from 3 pigs, Northern Ireland, February 2015.

Case	Isolation site[a]/Level of growth[b]	*Spa* type	MLST (*in silico*)	SCC*mec* type[c] (*in silico*)	*czrC*	*blaZ*	*Tet*(M)	*Tet*(K)	*lukM*	*lukF-P38*	*lukS/lukF-PV*	*tst*	EGC[d]	IEC[e]	Phenotypic resistance profile[f]
A	Brain/scant	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
B	Lung/scant	t1749	ST30	–	–	+	–	+	+	+	–	–	+	–	Pen Tet
	Brain/scant	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
	Joint hock/ moderate	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
	Skin/ profuse	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
C	Liver/scant	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
	Brain/scant	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
	Joint hock/ moderate	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet
	Skin/ profuse	t1749	ST30	Vt[c]	+	+	–	+	+	+	–	–	+	–	Pen Cef Tet

[a] *S. aureus* culture positive samples shown; remainder were culture negative.
[b] Scant = <10 cfu; Moderate = 50–100 cfu; Profuse = >200 cfu.
[c] SCCmec Vt based on the detection of mec gene complex C2 and ccrC genes C2 and C8.
[d] Enterotoxin gene cluster (*seg*,*sei*, *seu* positive).
[e] Immune evasion cluster (*scn, chp, sak, sea* negative).
[f] Pen = penicillin; cef = cefoxitin; tet = tetracycline; all isolates were susceptible to fusidic acid, ciprofloxacin, erythromycin, clindamycin, gentamicin, rifampicin, teicoplanin, vancomycin, daptomycin, linezolid and mupirocin.