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Letter to the Editor

MRSA USA300, USA300-LV and ST5-IV in pigs, Cuba

Sir,

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in humans represent a challenge for medicine and some clones belonging to sequence types ST1, ST8, ST80, ST59 and ST30 have been disseminating worldwide. Among them, ST8, also known as the USA300 clone, has been established as one of the major international epidemic strains causing infections both in community (CA-MRSA) and healthcare (HA-MRSA) settings [1]. It is characterised by the arginine catabolic mobile element (ACME) I, staphylococcal chromosome cassette *mec* (SCC*mec*) IVa and Panton–Valentine leukocidin (PVL). Another epidemic strain of CA-MRSA closely related to USA300 has emerged in northern South America and is known as the USA300-Latin American variant (USA300-LV). It differs from USA300 by SCC*mec* IVc and absence of ACME [1]. In 2011 in Cuba, human infections caused by MRSA were predominantly associated with USA300, and the Latin American variant was not identified [2]. Although these lineages are generally not associated with nasal carriage in pigs, which mainly harbour the livestock-associated MRSA (LA-MRSA) lineage ST398, the presence of human epidemic clones in pigs cannot be excluded. Since no data existed for LA-MRSA in Cuba, we decided to investigate the epidemiological MRSA situation in slaughter pigs from three Cuban provinces.

From May–July 2015, 22 of 285 nasal swabs taken at one central slaughterhouse from fattening pigs raised in the provinces of Mayabeque ($n = 67$), Matanzas ($n = 90$) and Cienfuegos ($n = 128$) were found to carry MRSA (prevalence 7.7%, 95% confidence interval 4.61–10.81%). Isolates were obtained on oxacillin resistance screening agar base (ORSAB) (Oxoid Ltd., Basingstoke, UK) supplemented with 3.5 mg/L cefoxitin after a two-step enrichment and selective cultivation in broth as previously described [3]. MRSA were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (microflex LT; Bruker Daltonics) and by detection of the methicillin resistance gene *mecA* by PCR [3].

The minimum inhibitory concentrations (MICs) of 19 antibiotics were determined by microdilution in Mueller–Hinton broth using the Sensititre susceptibility plate EUST (Thermo Fisher Scientific Oxoid Ltd., Basingstoke, UK) following guidelines and interpretation criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org). Antibiotic resistance and virulence genes, including those associated with innate immune evasion cluster (IEC) grouping [4], were detected using microarrays (*Staphylococcus* genotyping; Alere Technology, Waltham, MA). Mutations in the quinolone resistance-determining regions of GyrA and GrlA were detected as previously described [3]. Isolates were submitted to typing methods including pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *Staphylococcus* protein A (*spa*) typing, SCC*mec* typing, ACME typing and *mec*-associated direct repeat unit (*dru*) typing [5].

PFGE identified three major MRSA clonal clusters (I, II and III). All of the MRSA isolates gathering into PFGE cluster I contained the genetic properties specific to USA300 [all ST8 except one ST173 (a single *yqiL* allele variant), SCC*mec* IVa, *dru* dt9g, PVL+, ACME I+] and shared the same PFGE profile as the USA300 control strain (kindly provided by Jacques Schrenzel and Patrice François, Geneva University Hospitals) (Fig. 1). The isolates belonged to *spa* type t024 and also contained the *sek* and *seq* enterotoxin genes. The strains were resistant to β-lactams (*mecA*, *blaZ*), macrolides [*msrA*, *mph*(C)], aminoglycosides [*aph(3′)-III*] and fluoroquinolones [GrlA(S80-Y) and GyrA(S84-L)] (Fig. 1). MRSA clones of PFGE cluster II were characteristic to USA300-LV (ST8, *spa* t008, SCC*mec* IVc, *dru* dt7j, PVL+ and ACME–). These clones were resistant only to β-lactam antibiotics (*mecA*, *blaZ*), and only one single strain (Cuba7) contained the enterotoxin genes *seq* and *sek*, which are known to be either present or absent in the USA300-LV strains causing infections. The third PFGE cluster (cluster III) contained clones belonging to ST5, *spa* t010, SCC*mec* IVc, *dru* dt10a. They were all PVL– and ACME–, contained the enterotoxin genes *sea*(N315), *seb*, *sel*, *selm*, *seln*, *selo*, *egc* and *selu*, and were resistant to β-lactams (*mecA*, *blaZ*) as well as to the aminoglycosides gentamicin and kanamycin [*aac(6′)-Ie-aph(2″)-Ia*] (Fig. 1). A similar MRSA clonal lineage of ST5-SCC*mec* IVc, but harbouring PVL, has been associated with infections in paediatric patients in Latin America, but so far not in Cuba [1,2].

All of the MRSA strains identified in pigs also belonged to one of the innate IEC groups, which characterises *S. aureus* strains based on their toxin profiles and which has been associated with an extremely high incidence of clinical infections in humans [4]. The USA300 and USA300-LV strains belonged to IEC group B, which is determined by the presence of staphylokinase (SAK), chemotaxis-inhibiting proteins (CHIPS) and staphylococcal complement inhibitors (SCN). The ST5 strains belonged to IEC group F that additionally carry enterotoxin A *sea*(N315) (=*sep*), which is conserved on the β-haemolysin (hlb)-converting bacteriophages (βC-φs) (Fig. 1).

Although the origin of these MRSA clones in the pig population from Cuba is unknown, their presence was not linked to pigs of a specific region of Cuba. Whilst ST5 was only detected in pigs from Cienfuegos, USA300 was found both in Cienfuegos and Matanzas, and USA300-LV was spread in all three regions (Fig. 1). Notably, this study revealed the pig husbandry of Cuba as an unsuspected reservoir of epidemic MRSA strains including those of the USA300 lineage, which have also been identified as primary causes of MRSA infections in humans in Cuba [2]. Even if USA300-LV has not so far been reported in humans in Cuba, its introduction into pig husbandry is not likely to be directly associated with trade of animals, since no pigs are known to have been imported from Latin America. The presence of clinically relevant toxins in successful epidemic clones may represent a major public health issue for the country. It is therefore of major public health importance to take

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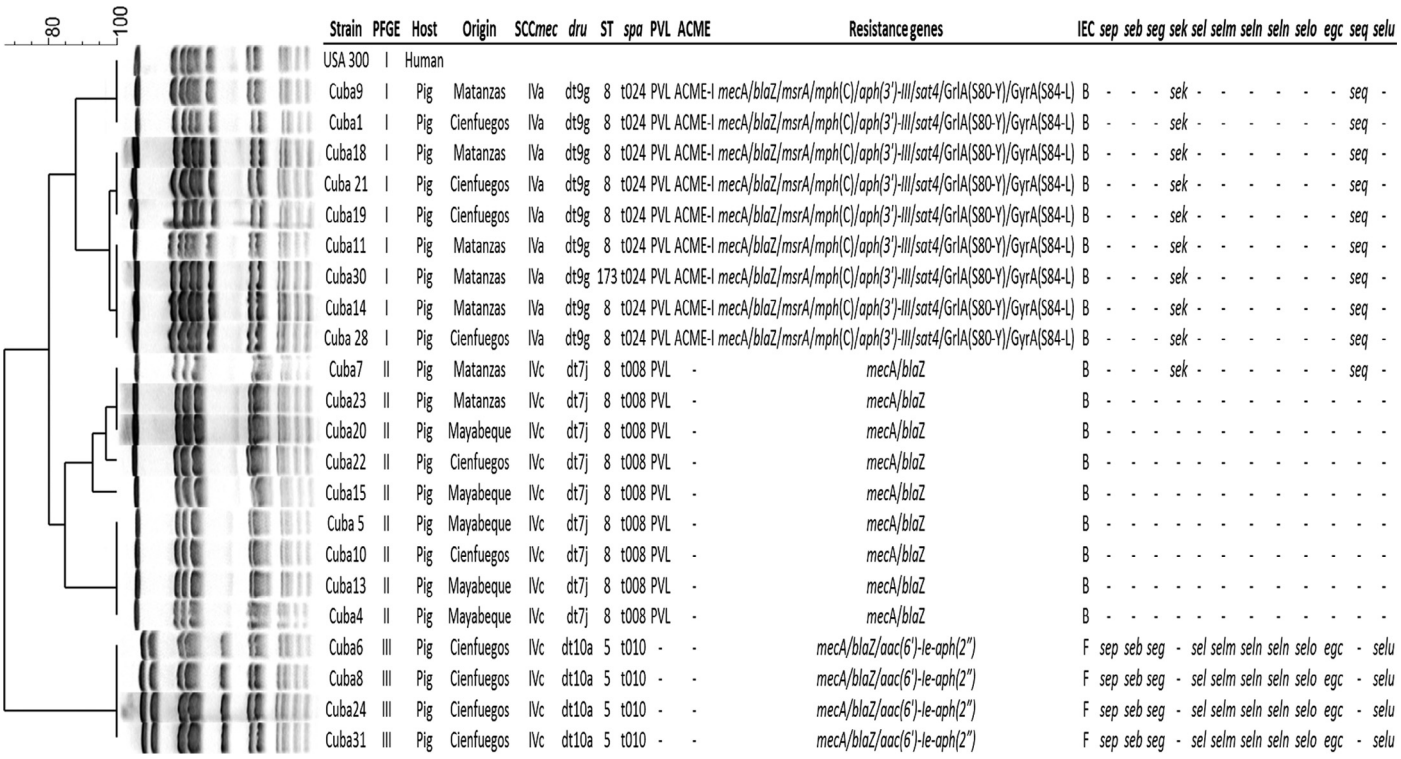

Fig. 1. Dendrogram of *Sma*I macrorestriction pulsed-field gel electrophoresis (PFGE) profiles and genetic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from slaughter pigs in three Cuban provinces. The tree was generated using BioNumerics software v.7.6 (Applied Maths, Sint-Martens-Latem, Belgium). The definition of a PFGE cluster (I, II and III) was based on a similarity cut-off of 80% [Dice coefficient, represented by UPGMA (unweighted pair-group method with arithmetic mean), 1.5% optimisation and 1% tolerance]. SCC*mec*, staphylococcal cassette chromosome *mec*; *dru*, direct repeat units [dt9g (5a-4a-0-2d-5b-3a-2g-3b-4e), dt7j (5a-3c-5b-3a-2g-3b-4e) and dt10a (5a-2d-4a-0-2d-5b-3a-2g-3b-4e)]; ST, sequence type (new sequence type ST173 has a simple change in *yqiL* allele 3-3-1-1-4-4-6); *spa*, *Staphylococcus* protein A gene; PVL, Panton–Valentine leukocidin; ACME I, arginine catabolic mobile element I (positive for the *arc* and *opp*3 genes). Resistance genes and function: *mecA*, penicillin-binding protein PBP2a; *blaZ*, β-lactamase; *msrA*, macrolide–streptogramin B efflux pump; *mph*(C), macrolide phosphotransferase; *aph(3′)-III*, aminoglycoside phosphotransferase for neomycin and kanamycin resistance; *aac(6′)-Ie–aph(2″)-Ia*, bifunctional aminoglycoside acetyltransferase and phosphotransferase for gentamicin, tobramycin and kanamycin resistance; *sat4*, streptothricin acetyltransferase; GrlA(S80-Y), DNA topoisomerase IV with a serine to tyrosine substitution in the quinolone resistance-determining region (QRDR) at position 80; GyrA(S84-L), DNA gyrase A with a serine to leucine substitution in the QRDR at position 84; *sak*, staphylokinase; *chp*, chemotaxis-inhibiting protein (CHIPS); *scn*, staphylococcal complement inhibitor; innate immune evasion cluster (IEC) B, *sak+*, *chp+*, *scn+*; IEC F, *sak+*, *chp+*, *scn+*, *sep+*. Enterotoxins: *sep* [=*sea*(N315)], enterotoxin P (=enterotoxin A allele from N315); *seb*, enterotoxin B; *seg*, enterotoxin G; *sek*, enterotoxin K; *sel*, enterotoxin L; *selm*, enterotoxin-like gene/protein M; *seln*, enterotoxin-like gene/protein N; *selo*, enterotoxin-like gene/protein O; *egc*, enterotoxin gene cluster; *seq*, enterotoxin Q; *selu*, enterotoxin-like gene/protein U.

adequate measures to limit the spread of the life-threatening bacteria both in the pig and human populations.

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