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Letter to the Editor

Meticillin-resistant *Staphylococcus aureus* in pigs from Thailand

Sir,

Livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) strains are increasingly isolated from pigs and humans, particularly those involved in pig farming. There is also growing evidence of transmission through the food chain. The distribution of LA-MRSA has spread throughout Europe and beyond, with the majority of strains belonging to sequence type ST398 and ST9. Here we report the emergence of LA-MRSA in pigs from Thailand.

Nasal and faecal swabs were taken from 10 healthy weaning pigs from four farms (total of 40 pigs) in Lampoon Province, Thailand. Swabs were enriched in brain–heart infusion broth containing 7% NaCl and were incubated at 37 °C for 48 h prior to inoculation onto mannitol salt agar supplemented with 4 μg/mL oxacillin. One farm yielded presumptive MRSA colonies from four pigs; three pigs with MRSA-positive nasal swabs and one pig yielding MRSA both from nasal and faecal swabs. All other swabs were culture-negative for MRSA. Biochemical testing for coagulase production and utilisation of glucose and mannitol was used to confirm identity; genotypic confirmation was provided by polymerase chain reaction (PCR) amplification of the *S. aureus*-specific thermonuclease gene *nuc* and *mecA* [1]. Multilocus sequence typing (MLST) determined that the isolates belonged to ST9, not a common type amongst human clinical isolates in Thailand. *spa* typing was employed to discern clonal lineages further; all five isolates were found to belong to *spa* type t337 (*spa* repeat profile 07-16-23-23-02-12-23-02-34). Recent publications documenting the occurrence LA-MRSA in Hong Kong [2] and Malaysia [3] also identified ST9. However, these isolates belonged to *spa* types t899 (07-16-23-02-34) and t4358 (07-16-23-02-02-34), both of which are similar to t337. Interestingly, reports describing *S. aureus* colonisation of pigs in Europe have found that ST9/t337 isolates rarely carry *mecA*; indeed, there have been few accounts of ST9/t337 MRSA, with only 4 of 52 isolates submitted to an online database being meticillin-resistant (http://www.spaserver.ridom.de/), whilst staphylococcal cassette chromosome *mec* (SCC*mec*) carriage by t899 and t4358 is common (http://www.spaserver.ridom.de/). SCC*mec* typing [4] revealed the presence of *ccrAB* type 1 and *mec* class C. This combination does not correspond to any currently described SCC*mec* type; *ccr* type 1 is only found in SCC*mec* type I, whereas *mec* class C is only found in SCC*mec* type V. This novel rearrangement of SCC*mec* may suggest recent acquisition of SCC*mec* by this lineage, possibly following its importation into Thailand, as this differs from the SCC*mec* types described in the Hong Kong and Malaysian LA-MRSA isolates. To determine the potential risk of ST9/t337 infection to human health, carriage of toxin genes was assessed. The staphylococcal enterotoxins SEG and SEI were found in all isolates, whereas genes for SEA-SEE and SEK, exfoliative toxins ETA and ETB, the Panton–Valentine leukocidin (PVL) and toxic shock syndrome toxin TSST-1 were not detected. This suggests that these isolates are unlikely to be responsible for any outbreaks of food poisoning. However, SEG and SEI are associated with gastrointestinal disease in human neonates [5], so their carriage by MRSA in food animals may be of concern in susceptible individuals. In addition, the presence of SEG and SEI indicates carriage of the enterotoxin gene cluster *egc*, which is found in the majority of successful MRSA clones; this has been suggested to confer higher fitness in staphylococci and may have contributed to the apparent global spread of ST9 in pigs.

Despite their apparent clonality, there was some variation in the antibiotic resistance profiles of the isolates (Table 1). Four of the five isolates were sensitive to sulfamethoxazole/trimethoprim, but one was resistant; three isolates were erythromycin-resistant whereas two were sensitive. This suggests that the ST9/t337 MRSA population has been established on the farm for sufficient time to evolve variation in resistance phenotype. Additionally, all isolates were highly resistant to chloramphenicol (minimum inhibitory concentration of 16 μg/mL), although the *cfr* gene conferring resistance to chloramphenicol, florfenicol and cross-resistance to linezolid, as previously identified in LA-MRSA, was not identified. This suggests that chloramphenicol resistance was conferred by the non-mobile chloramphenicol acetyl transferase (CAT), which does not cause cross-resistance.

This is the first report of pig-associated MRSA in Thailand. Carriage of a novel SCC*mec* arrangement is probably indicative of its de novo acquisition, highlighting the threat that new antibiotic-resistant pathogens may emerge from further unexpected sources.

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Table 1
Antibiotic resistance profiles of ST9/t337 livestock-associated meticillin-resistant *Staphylococcus aureus* (LA-MRSA) isolates by the disk diffusion test.

Isolate	Inhibition zone diameter (mm) (susceptibility)							MIC (μg/mL)	
	FLU (30 μg)	SXT (25 μg)	CLI (2 μg)	ERY (15 μg)	PEN (10 μg)	FOS (50 μg)	VAN (5 μg)	OXA	CHL
N3	15 (R)	6 (R)	6 (R)	6 (R)	10 (R)	34 (S)	14 (S)	16	16
N7	14 (R)	25 (S)	6 (R)	6 (R)	9 (R)	33 (S)	14 (S)	16	16
N10	16 (R)	25 (S)	6 (R)	6 (R)	11 (R)	27 (S)	16 (S)	32	16
N4	15 (R)	20 (S)	9 (R)	25 (S)	10 (R)	18 (S)	15 (S)	16	16
F4	15 (R)	17 (S)	9 (R)	28 (S)	10 (R)	34 (S)	14 (S)	16	16

ST, sequence type; MIC, minimum inhibitory concentration; FLU, flucloxacillin; SXT, sulfamethoxazole/trimethoprim (23.75 μg/1.25 μg); CLI, clindamycin; ERY, erythromycin; PEN, penicillin; FOS, fosfomycin; VAN, vancomycin; OXA oxacillin, CHL chloramphenicol; N, nasal isolate; F, faecal isolate; R, resistant; S, susceptible.

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