Investigation of meticillin-resistant *Staphylococcus aureus* in pigs used for research

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Meticillin-resistant *Staphylococcus aureus* (MRSA) was unexpectedly isolated from a pig used for streptozotocin-induced diabetes research. To investigate the possible source of the MRSA isolate, nasal swabs were obtained from the animal herd, and from animal holding rooms, and veterinary and research staff involved in the handling of the animals. Overall, four MRSA isolates were cultured from three pigs and from a clinician/scientist. Two were ST22-MRSA-IV, a human strain type associated with epidemic spread. The other two were ST398-MRSA-V, a strain type associated with pigs. Thus, care should be taken to prevent cross-transmission of MRSA.

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INTRODUCTION

Meticillin was introduced into clinical practice in 1960 and meticillin-resistant *Staphylococcus aureus* (MRSA) was described 1 year later (Jevons, 1961). Since then, MRSA has become a major human pathogen, responsible for considerable mortality, morbidity and healthcare expenditure in both nosocomial and community settings (Chambers, 2001; Fey *et al.*, 2003; Haddadin *et al.*, 2002).

Although rarely reported in the past, the prevalence of MRSA in pigs, along with cases of possible pig-to-human transmission and vice versa, have been the subject of considerable and increasing interest over the past 2 years (Armand-Lefevre *et al.*, 2005; Huijsdens *et al.*, 2006; Voss *et al.*, 2005).

In August 2005, MRSA was unexpectedly isolated from a pig used for streptozotocin-induced diabetes research in the Department of Experimental Surgery, Singapore General Hospital. The animal had a skin abscess from a surgical incision site and was administered enrofloxacin to treat infection. Unfortunately, the animal failed to respond to treatment, developed sepsis, lost 20 % of its body weight and was euthanized. MRSA was isolated from a bile specimen sent for bacterial culture taken post-mortem.

To investigate the possible source of the MRSA isolate and to determine whether there was an outbreak in the animal research facility, nasal swabs were obtained from the

animal herd and animal holding rooms, and from veterinary and research staff involved in the handling of the animals. Samples were also taken from the Animal Laboratory Centre of the National University of Singapore and from a slaughterhouse that was housing pigs of the same source as the department.

METHODS

Samples. Samples were taken from a Landrace/Yorkshire crossbreed of mixed ages and sex (40–180 kg). Nasal swabs were obtained from the animal herd ($n=50$) and animal holding rooms ($n=14$) of the Singapore General Hospital, Department of Experimental Surgery, Sembawang Farm and the Animal Research Facility, National University of Singapore Animal Laboratory Centre ($n=8$), and the slaughterhouse of Primary Industries Pte Ltd, Singapore ($n=50$). Human nasal swab isolates were obtained from veterinary staff ($n=23$), research staff ($n=6$) and animal laboratory staff ($n=3$) (see Table 2).

Experimental procedures. Nasal swab isolates were plated on to MRSA screening plates containing Mueller–Hinton agar with 6 µg oxacillin ml⁻¹ and 4 % NaCl. Bacteria were identified by positive slide and tube coagulation tests, the API Staph system, and universal 16S rRNA gene amplification and sequencing. Susceptibility to ciprofloxacin, erythromycin, clindamycin, tetracycline, gentamicin, rifampicin and vancomycin was tested using the Kirby–Bauer disc diffusion method and following the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) guidelines (NCCLS, 2005). The staphylococcal chromosome cassette *mec* (SCC*mec*) type was determined using methods described by Ito *et al.* (2004). All MRSA isolates were similarly typed by PFGE with *Sma*I using standard methods, and multilocus sequence typing (MLST) was performed as described by Enright *et al.* (2000). To determine the relationship of the MRSA

Abbreviations: MLST, multilocus sequence typing; MLVA, multilocus variable number tandem repeat analysis; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*.

Table 1. Antibigram of the recovered MRSA from pigs and a clinician scientist

MRSA	ST	PFGE profile	SCCmec type	Antimicrobial susceptibility						
				CIP	E	CC	TE	GM	RFB	VA
Pig 1	398	N	V	R	S	R	R	S	S	S
Clinician scientist	22	B	IV	R	R	Ri	S	S	S	S
Pig 2	22	B	IV	R	R	Ri	S	S	S	S
Pig 3	398	N	V	R	R	R	R	S	S	S

ST, Sequence type; CIP, ciprofloxacin; E, erythromycin; CC, clindamycin; TE, tetracycline; GM, gentamicin; RFB, rifampicin; VA, vancomycin; R, resistant; Ri, intermediate resistant; S, susceptible.

isolates to clinical isolates in Singapore, multilocus variable number tandem repeat analysis (MLVA) was performed (Sabat *et al.*, 2003). Virulence gene profiling was also carried out to assess the risk of human infection posed by the isolates (Jarraud *et al.*, 2002).

RESULTS AND DISCUSSION

The first pig MRSA isolate was resistant to clindamycin and ciprofloxacin, but susceptible to erythromycin and gentamicin (Table 1). This isolate possessed SCCmec type V. Following PFGE with *Sma*I, the isolate produced a smear across the gel lane rather than discrete bands, which is consistent with the description of non-typable *S. aureus* by Voss *et al.* (2005) and Huijsdens *et al.* (2006). The MLVA results did not match any of the other MRSA isolates in our national database. However, the MLST results revealed that the isolate belonged to ST398 (3-35-19-2-20-26-39). This sequence type is unique in Singapore and, along with the unusual antibiogram, suggested that the organism was likely to have originated from the pig rather than from a human source. Virulence gene profiling also revealed that the first pig MRSA isolate only possessed one toxin gene, γ -haemolysin, and was negative for all other toxin genes.

Among 93 isolates cultured during the initial sampling of pigs and humans, 1 pig imported from a commercial farm in Pulau Bulan, Indonesia, and a clinician scientist tested positive (Table 2). Although the two were never in direct contact, the results showed that the isolate was identical and belonged to ST22-MRSA-IV, also known as UK-EMRSA-15. A study on the dissemination of multisusceptible MRSA (susceptible to aminoglycosides, trimethoprim-sulfamethoxazole and tetracycline) in Singapore hospitals has revealed that this strain has recently been increasing in the hospital population (Hsu *et al.*, 2005).

As the two ST22 isolates were unrelated and independent of the first case of MRSA, and both pigs with MRSA had been imported from a commercial farm in Pulau Bulan, Indonesia, further sampling was performed in the Animal Laboratory Centre of the National University of Singapore and in the slaughterhouse. Both sites were housing pigs from the same source. Of the total 58 nasal swab isolates from pigs and 3 from humans, 1 pig was positive for MRSA whilst none of the human samples were positive (Table 2).

Upon typing, the isolate matched the SCCmec type, MLVA type and MLST of the first pig case, but had a slightly different antibiogram. The latter was resistant to erythromycin and susceptible to gentamicin, whilst the former was susceptible to both drugs (Table 1).

Overall, four MRSA isolates were identified: three from pigs from Pulau Bulan, Indonesia, and one from a clinician/scientist involved in the handling of the first pig case of MRSA.

Although the exact source of the first clinical isolate was never fully determined, it is likely to have originated from pigs imported from Indonesia, and did not seem to have spread to other pigs or humans in the department. The first and third strain isolated belonged to ST398, which is quite rare. There are two records in the MLST database, a meticillin-sensitive *S. aureus* (MSSA) isolate from a human nasal exudate in Cape Verde in 1997 and an MRSA isolate

Table 2. Results of MRSA and MSSA isolates from pigs and humans

	No. of samples (n)	No. of MSSA	No. of MRSA
Initial sampling			
Pigs			
Animal herd	50	0	0
Animal holding rooms	14	0	1
Humans			
Veterinary staff	23	8	0
Researchers			
Clinician scientist	2	1	1
Research staff	4	0	0
Total	93	9	2
Second sampling			
Pigs			
Animal lab centre	8	0	0
Slaughterhouse	50	0	1
Humans			
Animal laboratory staff	3	0	0
Total	61	0	1

from a human source in The Netherlands in 2003 (www.mlst.net).

Recently, reports in France and The Netherlands have shown the presence of MRSA in pigs, pig farmers and human controls (Armand-Lefevre *et al.*, 2005; Huijsdens *et al.*, 2006). Although some MLST sequence types were also found in human controls, ST398 was among those found only in pigs and pig farmers. It therefore seems likely that there is an association of this particular MLST type with pigs.

However, there was an implication that the second case of MRSA (ST22) in the pig could have been acquired from a human source. The pig had only been in contact with the veterinary staff of the department's holding unit and none of the other staff or researchers. Although all veterinary staff tested negative, it is possible that a clinician scientist who had the same strain as the pig could have contaminated the area. Clinician scientists move in and out of the facility and are distinct from the regular staff. Unfortunately, this could not be confirmed, as there were no samples collected from the departments' procedure areas and operating theatres. However, recent reports in veterinary hospitals suggest that, if MRSA is predominant in the environment, there is a risk of infection (Weese *et al.*, 2004). Thus, the pig could have acquired the infection whilst undergoing a procedure.

The reasons for the emergence of MRSA in the swine population remain uncertain. Recent publications proposed that there is a high risk of exposure for humans working with pigs and it is possible that strain exchange occurs between them on a large scale (Armand-Lefevre *et al.*, 2005; Huijsdens *et al.*, 2006; Voss *et al.*, 2005). It could also be due to increased exposure of animals to MRSA-infected people (Loeffler *et al.*, 2005; Weese *et al.*, 2005).

The isolation of the three strains from different pigs provides evidence that MRSA is a problem not only in hospitals but also in the veterinary field. It demonstrates that possible risks are involved in pigs used for experimental studies, as they may be potential recipients and sources of MRSA. This also has wider implications for research and for the development of treatment modalities in humans, as pigs are widely used in many types of biomedical research.

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REFERENCES

- Armand-Lefevre, L., Ruimy, R. & Andreumont, A. (2005). Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls and pigs. *Emerg Infect Dis* **11**, 711–714.
- Chambers, H. F. (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* **7**, 178–182.
- Enright, M. C., Day, N. P. J., Davies, C. E., Peacock, S. J. & Spratt, B. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* **38**, 1008–1015.
- Fey, P. D., Salid-Salim, B., Rupp, M. E., Hinrichs, S. H., Boxrud, D. J., Davis, C. C., Kreiswirth, B. N. & Schlievert, P. M. (2003). Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **47**, 196–203.
- Haddadin, A. S., Fappiano, S. A. & Lipsett, P. A. (2002). Methicillin resistant *Staphylococcus aureus* (MRSA) in the intensive care unit. *Postgrad Med J* **78**, 385–392.
- Hsu, L.-Y., Koh, T. H., Singh, K., Kang, M. L., Kurup, A. & Tan, B. H. (2005). Dissemination of multisusceptible methicillin-resistant *Staphylococcus aureus* in Singapore. *J Clin Microbiol* **43**, 2923–2935.
- Huijsdens, X. W., van Dijke, B. J., Spalburg, E., van Santen-Verheue, M. G., Heck, M. E. O. C., Pluister, G. N., Voss, A., Wannet, W. J. B. & de Neeling, A. J. (2006). Community-acquired MRSA and pig farming. *Ann Clin Microbiol Antimicrob* **5**, 26.
- Ito, T., Ma, X. X., Takeuchi, F., Okuma, K., Yuzawa, H. & Hiramatsu, K. (2004). Novel type staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. *Antimicrob Agents Chemother* **48**, 2637–2651.
- Jarraud, S., Mougé, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., Nesme, X., Etienne, J. & Vandenessch, F. (2002). Relationship between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles) and human disease. *Infect Immun* **70**, 631–641.
- Jevons, M. P. (1961). Celbenin-resistant staphylococci. *BMJ* **i**, 124–125.
- Loeffler, A., Boag, A. K., Sung, J., Lindsay, J. A., Guardabassi, L., Dalsgaard, A., Smith, H., Stevens, K. B. & Lloyd, D. H. (2005). Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* **56**, 692–697.
- NCCLS (2005). *Performance Standards for Antimicrobial Susceptibility Testing*, 14th informational supplement, M100-S15. Wayne, PA: National Committee for Clinical Laboratory Standards.
- Sabat, A., Krzyszton-Russjan, J., Strzalka, W., Filipek, R., Kosowska, K., Hryniewicz, W., Travis, J. & Potempa, J. (2003). New method for typing *Staphylococcus aureus* strains: multiple-locus variable tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J Clin Microbiol* **41**, 1801–1804.
- Voss, A., Loeffler, F., Bakker, J., Klaassen, C. & Wulf, M. (2005). Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis* **11**, 1965–1966.
- Weese, J. S., Dacosta, T., Button, L., Goth, K., Ethier, M. & Boehnke, K. (2004). Isolation of methicillin-resistant *Staphylococcus aureus* from the environment in a veterinary teaching hospital. *J Vet Intern Med* **18**, 468–470.
- Weese, J. S., Archambault, M., Willey, B. M., Dick, H., Hearn, P., Kreiswirth, B. N., Said-Salim, B., McGeer, A., Lkhioshvay, Y. & other authors (2005). Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. *Emerg Infect Dis* **11**, 430–435.