

## Prevalence of ST9 Methicillin-Resistant *Staphylococcus aureus* among Pigs and Pig Handlers in Malaysia<sup>∇</sup>

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Received 13 July 2009/Returned for modification 24 August 2009/Accepted 29 September 2009

**Methicillin-resistant *Staphylococcus aureus* (MRSA) of sequence type 398 (ST398) has frequently been detected in pigs and pig handlers. However, in Malaysia, sampling 360 pigs and 90 pig handlers from 30 farms identified novel ST9-*spa* type t4358-staphylococcal cassette chromosome *mec* type V MRSA strains that were found to transiently colonize more than 1% of pigs and 5.5% of pig handlers.**

Methicillin-resistant *Staphylococcus aureus* (MRSA) primarily causes human diseases and has recently been identified in pigs and pig handlers. This raised concerns about the role of this porcine reservoir in human infections. In Malaysia, several studies reported the prevalence and characteristics of MRSA isolates from clinical and community settings (15, 20). However, no data have yet been presented on MRSA in pigs. Here, we determined the prevalence of MRSA colonization in pigs and pig handlers in Malaysia.

Thirty randomly selected farms in the district of Kuala Langat from the Selangor state of Malaysia were sampled for MRSA. All farms were within a 5-km radius. The farm sizes ranged from 2,000 to 10,000 pigs. Twelve samples (three each from four different age groups, sows, piglets, weanlings, and grower-finishers) were taken at each farm. Nares of the pigs were swabbed by a trained veterinarian. In addition, the three pig handlers per farm (the maximum number of workers in a farm varied from six to eight) who had the highest level of exposure to pigs provided nasal swabs. Employees filled out a questionnaire regarding possible risk factors for MRSA colonization (11). Risk factors included the number of years of work with pigs on that farm, number of hours working with pigs per week, contact with other animals, recent hospitalization, recent treatment with antibiotics, personal or familial skin and soft tissue infection in the last 3 months, and participation in team sports.

MRSA was isolated according to methods described previously (13). The antibiotic susceptibility of the strains against the antibiotics mentioned below was tested by using the Kirby-Bauer disk diffusion method. The results of susceptibility testing were interpreted according to accepted guidelines (4). MRSA isolates were PCR tested for the *mecA* gene and subjected to staphylococcal protein A gene (*spa*) sequencing (<http://spaserver.ridom.de>), staphylococcal cassette chromosome

*mec* (SCC*mec*) typing (22), and multilocus sequence typing (<http://www.mlst.net>). All isolates were screened for the virulence genes *pvl* (14), *fnb* and *cna* (1), *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *tsst*, *eta*, and *etb* (8).

MRSA was isolated from one or more pigs on 30% (9/30) of the farms. The overall prevalence of MRSA among pigs was found to be 1.38% (5/360), with 5.3% (4/75) in weanlings and 1.3% (1/75) in grower-finishers. None of the piglets or sows was colonized. Except for farm 1, in all other eight farms, only one animal was colonized. The prevalence of MRSA colonization in humans was 5.5% (5/90). None of the risk factors identified in the questionnaire were found to have a significant association with human colonization. MRSA was not isolated from both pigs and pig handlers on any of the farms. When MRSA-positive animals were tested a second time, no MRSA was isolated. Unfortunately, no secondary samples were taken from humans. This indicates that MRSA is only transiently present, similar to what we found in a study with healthy Malaysians (16).

Susceptibility testing revealed 100% resistance to erythromycin, ceftriaxone, ceftiofur, ciprofloxacin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, clindamycin, and quinupristin-dalfopristine, while tigecycline, cephalixin (cefalexin), and fusidic acid showed 80%, 70%, and 20% resistance, respectively. No resistance was observed for mupirocin, amikacin, linezolid, vancomycin, and netilmicin. The surprising pattern of resistance to clindamycin, quinupristin-dalfopristine, and tigecycline was confirmed after three repeats of the test.

Molecular typing showed that MRSA isolates belonged to two sequence types: ST9 (*spa* type t4358) and ST1 (*spa* type t1784). Except for strains from handlers on farm 12 and 29 (ST1), all other isolates were ST9 (Table 1). All isolates carried SCC*mec* V. Virulence gene analysis revealed enterotoxin genes, such as *seb* (60%), *see* (10%), and *seg* (90%), and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that include *Cna* (20%, only in ST1 isolates) and *Fnb* (100%). None of the MRSA isolates carried *pvl*, *eta*, *etb*, *tsst*, or enterotoxin genes other than *seb*, *see*, and *seg* (Table 1).

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<sup>∇</sup> Published ahead of print on 7 October 2009.

TABLE 1. Characteristics of MRSA isolates from pigs and pig handlers in Malaysia

No.	Sample code <sup>a</sup>	SCC <sub>mec</sub>	<i>spa</i> type	Presence or absence of virulence gene												
				<i>cna</i>	<i>fnb</i>	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>	<i>see</i>	<i>seg</i>	<i>seh</i>	<i>eta</i>	<i>etb</i>	<i>tsst</i>	<i>pvl</i>
1	F1 W2	V	t4358	-	+	-	-	-	-	+	+	-	-	-	-	-
2	F1 W3	V	t4358	-	+	-	-	-	-	-	+	-	-	-	-	-
3	F8 W3	V	t4358	-	+	-	+	-	-	-	+	-	-	-	-	-
4	F10 W2	V	t4358	-	+	-	+	-	-	-	+	-	-	-	-	-
5	F12 L1	V	t1784	+	+	-	-	-	-	-	-	+	-	-	-	-
6	F14 L2	V	t4358	-	+	-	-	-	-	-	-	+	+	-	-	-
7	F23 GF1	V	t4358	-	+	-	+	-	-	-	+	-	-	-	-	-
8	F25 L2	V	t4358	-	+	-	+	-	-	-	-	+	+	-	-	-
9	F29 L1	V	t1784	+	+	-	+	-	-	-	-	+	+	-	-	-
10	F29 L3	V	t4358	-	+	-	+	-	-	-	-	+	+	-	-	-

<sup>a</sup> F, farm; L, labor; W, weanling pigs; GF, grower finisher.

Although a previous study from Malaysia (10) has documented MRSA colonization in pigs, the strain types were not characterized. A recent study (5) from China reported ST9 as a dominant strain among pigs and pig handlers; however, the isolates were of SCC<sub>mec</sub> type III and *spa* type t899. To the best of our knowledge, our study reports the isolation of MRSA ST9-t4358-SCC<sub>mec</sub> V from pigs and humans in the Asian region for the first time. The results from the current study show that MRSA colonization among pigs (1.38%) and pig handlers (5.5%) is lower in Malaysia than in the United States (21), The Netherlands (6), Canada (11), and Denmark (13). Our colonization rate is in agreement with the results of a previous study from our country (0.8%) (10). In contrast to most reports, our isolates were found to be ST9 and not the more common ST398. Methicillin-susceptible *S. aureus* (MSSA) ST9 strains were previously reported to be isolated from pig farmers and also from swine infections in France and Germany (2, 9). MSSA ST9 strains have occasionally been isolated from healthy human carriers (7). The genetic background of ST9 identified in the current study has only been reported as MSSA until now, except for two ST9 (SCC<sub>mec</sub> type unknown) MRSA isolates obtained in Miami hospitals, with no association with pigs (3). The fact that the MSSA genetic background was identified in MRSA strains in the Miami hospitals, as well as in the current study, is consistent with the notion of the relatively frequent acquisition of the SCC<sub>mec</sub> element by *S. aureus*. The current study provides further support to the hypothesis that MRSA can be transmitted between humans and pigs, as was previously observed for ST398 (2).

We show colonization by ST1-t1784-SCC<sub>mec</sub> V MRSA strains in pig handlers. A recent study by Otter and French (18) reported ST1-SCC<sub>mec</sub> IV MRSA as a cause of infection in the homeless and injection drug users. The differences between these and our isolates are the replacement of SCC<sub>mec</sub> IV with SCC<sub>mec</sub> V and the absence of the Pantone-Valentine leukocidin (PVL) gene. PVL-positive and -negative ST1 MRSA and MSSA strains have been detected among clinical strains in Malaysia (unpublished data), but these were of *spa* type t0127. The ST1 strain isolated in the current study may be related to USA400, but due to the differences noted (PVL negative and SCC<sub>mec</sub> V instead of IV), additional characterization is required to confirm the relatedness.

Multiresistant porcine MRSA strains have also been identified in many other countries (7, 13). Surprisingly, we observed

combined resistance against quinupristin-dalfopristine and tigeicycline among isolates of both STs. However, all farms where we sampled did not use quinupristin-dalfopristine or tigeicycline for prophylaxis or as therapeutics. Therefore, our data do not allow us to draw definite conclusions on the relationship between local antimicrobial use and the development of antimicrobial resistance. Virulence gene characterization showed that the majority of the isolates carry enterotoxin-encoding genes. As pigs are food-producing animals, there are inherent concerns about contamination of food. Pereira et al. (19) observed a higher incidence of different types of enterotoxins among isolates obtained from fermented meat products. The high prevalence of enterotoxigenic genes among the ST9 isolates is in contrast with swine-associated ST398 isolates, which are negative for enterotoxins (12). In addition, the higher incidence of enterotoxins, especially *seb* and *seh*, among pig isolates than among typical human isolates (17) gives warning that even though the MRSA prevalence in Malaysia is low, the toxigenic nature of the clone may pose a greater risk to humans via contact or through consumption of contaminated food. However, further characterization of the strains needs to be carried out to understand the virulence potential of the enterotoxin genes in ST9.

In conclusion, we report the first ST9 and ST1 MRSA isolates from pigs and pig handlers in Malaysia. Although the prevalence of MRSA is low outside Malaysian hospitals, the elevated incidence among pig handlers demonstrates the regional emergence of community-associated MRSA. The prevalence of MRSA in farm animals and handlers needs to be monitored continuously, as it may play a vital role in food safety and public health.

This work was supported by Escience fund 02-01-04-SF0853 from the Ministry of Science, Technology, and Innovation (MOSTI), Malaysia.

The authors declare no conflict of interest.

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