Prevalence of carriage and characterisation of methicillin-resistant *Staphylococcus aureus* in slaughter pigs and personnel exposed to pork carcasses

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KEY MESSAGES

1. There is a high level of contamination of pig carcasses in Hong Kong with multi-drug resistant methicillin-resistant *Staphylococcus aureus* (MRSA) ST9.
2. Personnel occupationally exposed to pigs and pig carcasses are at risk of colonisation and possible infection.
3. Whilst the clinical significance of porcine MRSA ST9 is unclear, there is a need for continued surveillance of this potential reservoir of MRSA.
4. Butchers should be encouraged to wear gloves whilst working and maintain good personal hygiene to reduce colonisation risk.

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Introduction

In recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged in the community. There have been increasing reports of MRSA colonisation of pigs and pig farmers in Europe[1] and North America. Multi-locus sequence typing (MLST) revealed widespread dissemination of a particular sequence type (ST) 398 among pigs in the Netherlands and subsequently the occurrence of this MRSA lineage has been reported in several other countries and animal species. Compelling microbiological and epidemiological evidence indicates that people who live or work on farms, especially pig farms, have an increased risk of colonisation or infection with ST398 MRSA. Human infection with ST398 MRSA has been reported in Hong Kong, but no information about occupation or pig contact was available.

Porcine-associated MRSA ST398 typically harbours Staphylococcal cassette chromosome (SCC) *mec* type IV or V, but unlike most community MRSA, ST398 is increasingly multi-drug resistant. It appears that the genes for Panton-Valentine leucocidin toxin are rare in these strains. ST398 is non-typable by pulsed field gel electrophoresis (PFGE) using the restriction endonuclease *sma*1, which is commonly used in the investigation of clonality of staphylococcal strains.

This study aimed to (1) determine the MRSA colonisation level of locally slaughtered pigs and whether this colonisation results in spread to persons occupationally exposed, (2) characterise any MRSA isolates for the presence of toxin mediating genes, and (3) compare ST398 MRSA strain in this locality with the predominant strain reported elsewhere.

Methods

This study was conducted from January 2009 to December 2012. Ethics approval was obtained from the Human Subjects Ethics Sub-Committee of The Hong Kong Polytechnic University. Nasal samples were collected from 400 slaughtered pigs after distribution to three wet markets in Hong Kong over a 3-month period. Butchers at all major wet markets in Hong Kong were invited to participate. A total of 300 butchers consented to sampling by self-collection of a nasal swab according to instructions. Butchers were asked about any recent hospitalisation, healthcare workers in the family, wound infections within the last year, and antibiotic use in the last 6 months. Nasal swabs were collected from 100 to 150 pigs of different ages (weaning pigs, market ready animals, and breeding sows) from two farms each in Hong Kong (n=220) and Guangdong (n=255). A small number of environmental samples were collected at the Hong Kong farms.

Swabs were enriched in brain heart infusion, cultured on selective agar and presumptive MRSA colonies were identified as *S aureus* by latex agglutination. Susceptibility to a range of antibiotics was determined following standard guidelines.

MRSA isolates were tested by PCR for the

*mec*A gene, resistance determinants for macrolides, tetracycline, and chloramphenicol and subjected to SCC*mec* typing and staphylococcal protein A gene (*spa*) sequencing (http://spaserver.ridom.de). All isolates were screened for genes for Panton-Valentine leucocidin, enterotoxins (*sea, see*), and exfoliative toxins. PFGE using *sma*1 digestion was performed on one representative sample from each of the antibiotic susceptibility patterns. Isolates representative of a distinct PFGE pattern and those of non-t899 spa types were analysed by MLST.

Results

MRSA was detected in 39.3% (95% confidence interval [CI]=38.8-39.8%) of slaughtered pigs and overall 170 MRSA strains were isolated and confirmed to harbour *mec*A, SCC*mec* types IVb (92%) or V (8%), and belong to *spa* type t899 or closely related variants; t4474 (2 isolates), and one isolate each of t1939, t2922, and t5390.

Seventeen (5.7%, 95% CI=4.2-7.0%) butchers were MRSA-colonised. Fifteen strains harboured SCC*mec* IV of which ten were t899 and belonged to ST9. The remaining type IV strains were t008 (ST8), t002 (ST5), and t123 (ST45), all of which have been reported from skin and soft tissue infections in Hong Kong, and single isolates of t359 (ST747) and t375 have been reported from buffaloes and nasal colonisation, respectively. Two strains were SCC*mec* II. These were healthcare-associated t701 (ST6) and were isolated from two workers at the same stall. None of the butchers had been recently hospitalised nor had a healthcare worker in their immediate family. Two of those colonised had received antibiotics in the last 6 months, one for a skin infection, and four reported a wound infection within the last year. All butchers were exposed to meat for at least 9 hours per day and none routinely wore gloves.

None of the samples collected at the Hong Kong farms yielded MRSA, but a high percentage of pigs at both mainland farms were colonised. The rate of colonisation of the pigs varied considerably between farms (Table 1). At farm 1 all isolates were t899 (ST9), but at farm 2 three isolates had *spa* types closely related to t899; t1334 (2 isolates) and t4358.

The majority of carcass isolates were multi-drug resistant (Table 2); 91% were resistant to four or more classes of non-beta-lactam antibiotics. The most predominant pattern (45% of isolates) included resistance to fluoroquinolones, tetracyclines, macrolides/lincosamides/streptogramins, and chloramphenicol. Resistance to fusidic acid and rifampicin was uncommon. ST9 isolates from butchers were much more resistant than non-livestock-associated strains (90% resistant to clindamycin, 80% resistant to chloramphenicol, quinupristin/dalfopristin, erythromycin, and tetracycline, 50% resistant to ciprofloxacin, 30% to fusidic acid, and 20% to cotrimoxazole and gentamicin). Resistance rates were quite similar in isolates from the two mainland farms although farm 2 was lower in terms of some agents (Table 3). Overall no isolates were resistant to linezolid, nitrofurantoin, or tigecycline.

All resistance to erythromycin was attributable to *erm*(C), and chloramphenicol resistance to *fex*(A). No chloramphenicol-resistant isolate was positive for *cfr* and there was no resistance to linezolid although MICs were close to the resistance breakpoint. All tetracycline-resistant strains carried *tet*(K), but 3% additionally carried *tet*(M). No virulence factor genes were detected. There was no relationship between SCC*mec* type and occurrence of antimicrobial resistance genes in pig isolates, but in human isolates there was more variation with some SCC*mec* type IV and V isolates displaying limited resistance, characteristic of typical community MRSA strains, whereas others were t899 and matched the pig isolates. Isolates found to be type II were resistant only to the beta-lactams.

PFGE of representative strains of carcass isolates revealed eleven different banding patterns by *sma*1digestion (Table 2). Cleavage of the DNA by *sma*1 suggested that the strain was not ST398. MLST subsequently confirmed that all isolates were ST9. The banding patterns showed that many of the strains were closely related, and this relationship was associated with the SCC*mec* type. PFGE of strains from live pigs revealed related strains. The banding patterns displayed by the human isolates varied much more, but those of t899 closely matched the porcine strains.

Discussion

Samples from the anterior nares of slaughtered pigs at three representative regional wet markets in Hong Kong demonstrated a high MRSA colonisation rate, which was comparable with the situation in the Netherlands and Belgium (~40%).[1] The colonisation rate in butchers was much higher than that reported for the general public in Hong Kong (~1%) and exceeded that of healthcare workers (3.2%). Of the

TABLE 1. Methicillin-resistant *Staphylococcus aureus* (MRSA) colonisation of live pigs at farms in Guangdong

Pig type	% (No. of MRSA positive/total No. sampled)	
	Farm 1	Farm 2
Weaners	73.5 (25/34)	34 (17/50)
Finishers	75.8 (25/33)	38 (19/50)
Sows	0 (0/38)	44 (22/50)
Total	47.6 (50/105)	38.6 (58/150)

TABLE 2. Susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) strains (n=170) isolated from pig carcasses

No. of strains	Market			Antimicrobial resistance*									SCC*mec* type	PFGE type	*spa* type
	Ngau Chi Wan (n=100)	Shatin Central (n=100)	Hung Hom (n=200)	C	Cip	Da	E	F	QD	R	Sxt	T			
37	5	5	27	+	+	+	+		+			+†	IVb	A	**t899**
26	0	6	20	+	+	+	+					+	IVb	A1	t899
14	1	5	8		+	+	+					+†	IVb	A2	t899
12	4	3	5	+	+	+	+		+		+	+†	IVb	A3	t899
10	5	1	4	+		+	+		+			+	V	F1	t1939
9	1	3	5	+	+	+	+				+	+	IVb	A1	t2922
9	0	4	5		+	+	+				+	+	V	F	t5390
7	1	3	3	+		+	+				+	+	IVb	A	t899
6	3	0	3		+	+	+		+			+	IVb	B	t4474, t899
4	0	2	2			+	+					+	IVb	A	t899
3	1	2	0			+						+	IVb	B	t899
3	1	0	2	+		+	+					+	IVb	A	t899
2	0	0	2		+	+						+	IVb	A3	t899
2	2	0	0	+	+	+		+			+	+	IVb	A	t899
2	1	0	1		+	+	+		+		+	+	IVb	B1	t4474
2	0	0	2	+	+	+						+	IVb	A	t899
2	0	1	1			+	+		+		+	+	IVb	A	t899
2	0	1	1	+		+						+	IVb	A	t899
2	0	1	1	+	+	+					+	+	IVb	A	t899
1	1	0	0		+							+	IVb	E	t899
1	0	0	1			+		+			+		IVb	A	t899
1	0	0	1			+		+				+	IVb	A	t899
1	0	1	0	+	+	+		+		+	+	+	IVb	A	t899
1	0	0	1	+	+	+	+	+			+	+	IVb	A	t899
1	0	0	1		+	+			+		+	+	IVb	A	t899
1	0	1	0	+	+	+	+		+		+		IVb	A1	t899
1	0	1	0		+	+	+				+		IVb	A	t899
1	1	0	0	+	+	+	+		+	+		+	IVb	A	t899
1	0	1	0	+	+	+	+			+		+	IVb	A	t899
1	1	0	0	+	+	+	+			+	+	+	IVb	A	t899
1	0	1	0	+	+	+	+						IVb	D	t899
1	0	0	1	+		+	+		+		+	+	IVb	A	t899
1	0	1	0			+	+				+	+	IVb	A	t899
1	0	1	0			+	+				+		V	C	t899
1	0	0	1			+	+		+			+	IVb	A	t899
No. (%) of MRSA strains resistant	28 (28)	44 (44)	98 (49)	120 (71)	133 (78)	169 (99)	152 (89)	6 (4)	74 (44)	4 (2)	55 (32)	165 (97)			

* C denotes chloramphenicol, Cip ciprofloxacin, Da clindamycin, E erythromycin, F fusidic acid, QD quinupristin-dalfopristin, R rifampicin, Sxt co-trimoxazole, and T tetracycline. No strains were resistant to vancomycin, linezolid, nitrofurantoin, or tigecycline

† *tet* (M) was found in strains of these three groups (3/37, 1/14, and 1/12, respectively)

colonised butchers, the highest proportion carried ST9 (3.3%). It was only possible to sample these workers on one occasion; it would be useful to determine whether the colonisation was persistent. Persistence of ST398 colonisation in farmers depends on the intensity of contact.[2] All our subjects were exposed to meat for a minimum of 9 hours daily, with many working 7 days each week. Such

TABLE 3. Percentage resistance to antibiotics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from pigs at two farms in Guangdong

Farm	Antimicrobial resistance* (%)								
	C	Cip	Da	E	F	QD	CN	Sxt	T
1	44	84	100	98	7	91	49	3.5	96.5
2	32	100	100	100	5	57	25	5	93

* C denotes chloramphenicol, Cip ciprofloxacin, Da clindamycin, E erythromycin, F fusidic acid, QD quinupristin-dalfopristin, CN gentamicin, Sxt co-trimoxazole, and T tetracycline

levels of colonisation would pose an increased risk of MRSA infection.

Interestingly, MRSA was not isolated from pigs or environmental samples at either of the Hong Kong pig farms with 3000-5000 pigs and 3-4 employees. The pig accommodation was covered but not enclosed, allowing constant fresh air circulation. Both farms claimed to use no antibiotics except for individual sick animals that would be isolated during illness. Unfortunately, we were unable to gain access to any other farms in Hong Kong, so the absence of MRSA from all farms cannot be assumed. In mainland China, both farms visited were very large and comprised several enclosed barns with fan-assisted ventilation. The infection control precautions were strictly enforced. Nevertheless, MRSA was present on both farms at levels similar to that found for MRSA ST398-infected farms in the Netherlands (42%). The managers claimed that the pigs did not receive antibiotic-containing feed. Use of prophylactic antibiotics could not be confirmed, but use for sick animals was reported.

At the commencement of this study, it was expected that any porcine MRSA detected would most likely be ST398, the predominant porcine strain reported in Europe and North America. Nonetheless, although the isolates were t899, they were typable by PFGE with *sma*1. MLST revealed that the strains were ST9. Other researchers have reported the presence of MRSA ST9 on farms in mainland China, although their sampling was performed in different regions.[3]

Twelve animals from one of the Hong Kong farms were tracked through the slaughtering and butchering processes. Although negative before commencing their journey to the abattoir, they were all positive for MRSA after slaughter, suggesting cross-infection at the central abattoir. We requested to be allowed to sample in the abattoir but were denied access.

In contrast with European swine MRSA isolates in which SCC*mec* III, IVa, and V have been reported, only SCC*mec* IVb and V were present in our porcine isolates. This suggests relatively limited genetic diversity among Asian swine MRSA isolates, and horizontal transfer is not an important mechanism of transfer between strains. Although SCC*mec* types IV and V are typical of community MRSA, our ST9 strains were resistant to multiple classes of antibiotics, which is unusual in community MRSA. The high degree of resistance in porcine isolates suggests wide exposure to antibiotics, possibly used in pig husbandry. The major source of pigs for the Hong Kong market is Mainland China. Such multi-resistance to antibiotics used in human medicine could make infection with swine MRSA difficult to treat.

Tetracycline resistance in porcine isolates was mainly mediated by *tet*(K). Only a few porcine isolates harboured two tetracycline resistance determinants. In contrast, the majority of ST398 isolates from Germany harboured two determinants. Erythromycin resistance in porcine strains was mediated by *erm*(C). This finding is similar to that reported in Europe, although *erm*(A) and *erm*(B) have been reported in Germany. All human ST9 isolates also harboured *erm*(C).

Although virulence factors are not commonly found in swine MRSA, the high colonisation rate of pigs indicates a need for continued surveillance of this potentially large reservoir for human infections. This study has shown the potential for ST9 porcine strains to colonise humans exposed to pork meat. In addition, there is a need to look at possible transmission of these strains to humans via the food chain and via contact with persons working with pigs.

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