Isolation of meticillin-resistant *Staphylococcus aureus* (MRSA) from swine in Japan

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ABSTRACT

Meticillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 is widely prevalent in swine in Europe and North America. To determine the prevalence of MRSA, and specifically ST398, in Japanese swine, a total of 115 nasal swabs and 115 faecal samples from swine reared at 23 farms located in eastern Japan were investigated. MRSA was isolated from a nasal sample (0.9%) but not from any faecal samples. The strain of MRSA was classified as ST221 by multilocus sequence typing and as t002 by *spa* typing. The MRSA isolate exhibited resistance to ampicillin, meticillin and dihydrostreptomycin. Interestingly, it remained susceptible to cefazolin, ceftiofur, Imipenem, gentamicin, kanamycin, chloramphenicol, oxytetracycline, erythromycin, azithromycin, tylosin, vancomycin, enrofloxacin and trimethoprim. The prevalence of MRSA among swine was low and MRSA ST398 was not recovered in the present study.

1. Introduction

Since meticillin-resistant *Staphylococcus aureus* (MRSA) was isolated from humans working in close proximity to swine in The Netherlands, a number of studies have been conducted to determine the prevalence and characterisation of MRSA among swine, swine producers and their families [1–4]. MRSA isolates transmitted between swine and swine farmers were classified as sequence type (ST) 398 using multilocus sequence typing (MLST). MRSA ST398 is disseminated by colonised swine through swine production systems. According to data of the Animal Quarantine Service of the Japanese Ministry of Agriculture, Forestry and Fisheries (http://www.maff.go.jp/aqs/tokei/toukeinen.html), more than 100 heads of breeding swine are imported from the USA and Canada every year to Japan. In addition, during 2005 a total of 75 swine were imported from The Netherlands to Japan.

Some surveys on MRSA of swine origin have been conducted in Asian countries, for example in China, Malaysia and Singapore [5–7]. Interestingly, MRSA clonal complex (CC) 9 is predominantly isolated from swine in China and Malaysia [5,6]. In Singapore, MRSA ST398 was isolated from swine [7]. In Japan, although MRSA (MLST analysis was not done) was isolated from retail ground pork in 2005 [8], MRSA has not been isolated from swine. The objective of this study was to determine the prevalence of MRSA in swine in Japan.

2. Materials and methods

A total of 115 nasal swab samples and 115 faecal samples from swine reared on 23 swine farms in seven prefectures of Eastern Japan were collected at a

slaughterhouse between March and September 2009. Nasal and faecal samples were collected from five animals per farm. Nasal swabs were sampled from the nasal cavity using a sterile cotton swab. Faecal samples were collected using a medicine spoon and were placed in a sterilised sampling bag. One gram of each faecal sample was used for isolation.

Samples were inoculated into 9 mL of heart infusion broth (Difco Laboratories, Detroit, MI) containing 7.5% NaCl and were incubated for 18 h at 35°C. Following incubation, the enrichment culture was inoculated onto two commercial isolation agars, namely CHROMagar™ MRSA Medium (Kanto Chemical Co. Inc., Tokyo, Japan) and MRSA Selective Agar (Becton Dickinson & Co., Franklin Lakes, NJ). Suspected colonies of staphylococci were tested with oxidase (Nissui Co. Ltd., Tokyo, Japan), catalase and Gram stain. Subsequently, a tube coagulase test (Eiken Chemical Co. Ltd., Tochigi, Japan) and an N-ID test (Nissui Co. Ltd.) were conducted on isolates to identify *Staphylococcus* spp.

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed by amplification of the *mec* regions (classes A, B and C) and the *ccr* regions (types 1, 2 and 3) by multiplex polymerase chain reaction (PCR) using the primers described by Kondo et al. [9]. The genes of *mecI*, *ccrB2* and *ccrC* were additionally examined by PCR using the primers described by Milheiriço et al. [10]. The ST of the isolate was determined from the MLST website (http://saureus.mlst.net/) and the *spa* type was identified using the Ridom database website (http://spaserver2.ridom.de/index.shtml).

Minimal inhibitory concentrations of antimicrobials were determined using a broth dilution method following the guidelines of the Clinical and Laboratory Standards Institute [11,12]. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 served as quality control isolates. Susceptibility testing was conducted against ampicillin, oxacillin, cefazolin, ceftiofur, Imipenem, dihydrostreptomycin, gentamicin, kanamycin, chloramphenicol, oxytetracycline, erythromycin, azithromycin, tylosin, vancomycin, enrofloxacin and trimethoprim.

3. Results

MRSA was isolated from only 1 of 115 nasal samples (0.9%, 95% confidential interval 0.0–4.8%) (Table 1). MRSA isolates harboured the type 1 *ccr* complex. The sequence of the PCR product showed 87% homology with type 1 *ccr* complex of *S. aureus* PL72 strain (accession no. AB433542). However, no amplicon was detected by PCR for the *mec* complex or the *mecI*, *ccrB2* and *ccrC* genes.

The MRSA isolate was classified as ST221 (allelic profile 1-4-1-4-12-1-10) belonging to CC5, according to the MLST database. The *spa* type of the isolate was t002. The isolate was resistant to ampicillin, oxacillin and dihydrostreptomycin, but remained susceptible to the other 13 antimicrobials (Table 1).

4. Discussion

This study showed MRSA ST221 to be present in the nasal cavity of slaughtered swine in Japan. Since 2005, the high prevalence of MRSA ST398 in the nasal cavity of swine has been reported in some Europe and North America, with an isolation

rate between 24.9% and 49% [1–4]. Although many pigs have been imported from these countries to Japan, ST398 does not as yet seem to have appeared in Japan. It should be noted that animals are not quarantined for carriage of MRSA when imported because it is not a targeted infectious disease according to the Domestic Animal Infectious Disease Control Law (http://www.cas.go.jp/jp/seisaku/hourei/data/adaidc.pdf). Although this study utilised an enrichment culture method using broth medium containing 7.5% NaCl, it seems likely that the isolation rate of MRSA from swine in Japan (0.9%) was lower than those in Europe and North America.

The MRSA isolate belonging to CC5 and t002 was related to the New York/Japan MRSA clone with SCC*mec* II. However, the SCC*mec* type of the MRSA ST221 in this study was untypeable since the class of *mec* complex was not determined. Moreover, DNA homology of *ccr* type 1 of this MRSA isolate with previously reported isolates was low. Zaraket et al. [13] reported that the New York/Japan MRSA clone was predominant in Japan. Previously, a strain of MRSA ST221 with SCC*mec* II (*spa* type not determined) was isolated at a hospital between 2001 and 2003 in South Korea [14] and an isolate with SCC*mec* I and t149 was isolated at a hospital in 2005 in Paraguay [15]. The MLST database (http://saureus.mlst.net/sql/burstspadvanced.asp) showed that *S. aureus* ST221 was isolated at French and Scotland hospitals in 2002 and 2003, respectively. To the best of our knowledge, there is no previous report detailing the isolation of MRSA ST221 from animals. In addition, the MRSA ST221 isolate in this study exhibited resistance only to β-lactam antibiotics and dihydrostreptomycin (Table 2). The New York/Japan MRSA clone exhibited resistance not only to β-lactam antibiotics but also

aminoglycosides, macrolides, tetracycline and fluoroquinolones [13]. MRSA from swine showed resistance to tetracycline antibiotics in several Western countries [1]. MRSA isolated from ground pork in Japan in 2005 exhibited resistance to benzylpenicillin, ampicillin, cefazolin, oxacillin and oxytetracycline [8]. The resistance profile revealed that the isolate was considerably different from this MRSA clone. At present, the origin of MRSA ST221 infection in swine remains unclear.

This study was the first survey on the prevalence of MRSA in Japanese swine. At present, the prevalence of MRSA among swine in Japan is low and MRSA ST398 was not isolated from swine in this study.

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Competing interests

None declared.

Ethical approval

Not required.

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Table 1

Prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) in Japanese swine

Sample	No. of farms positive/tested (%, 95% CI)	No. of pigs positive/tested (%, 95% CI)
Nasal swab	1/23 (4.3, 0.1–22.0)	1/115 (0.9, 0–4.8)
Faeces	0/23 (0, 0–12.2)	0/115 (0, 0–2.6)

CI, confidential interval.

Table 2

Minimum inhibitory concentrations (μg/mL) for meticillin-resistant *Staphylococcus aureus* (MRSA) sequence type ST221 strain in this study as well as quality control strains in accordance with the Clinical and Laboratory Standards Institute (CLSI)

	Breakpoint [a]	MRSA ST221	*S. aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212
Ampicillin	0.5	>128	≤1	≤1
Oxacillin	4	256	0.25	16
Cefazolin	32	8	≤1	N/D
Ceftiofur	N/D	2	1	N/D
Imipenem	16	<1	<1	<1
Dihydrostreptomycin	32	>128	8	64
Gentamicin	16	1	≤0.5	16
Kanamycin	64	8	≤4	64
Chloramphenicol	32	8	8	4
Oxytetracycline	16	≤0.5	≤0.5	16
Erythromycin	8	0.5	0.5	2
Azithromycin	8	2	2	8
Tylosin	N/D	2	2	2
Vancomycin	16	2	2	4
Enrofloxacin	N/D	0.063	0.063	0.25
Trimethoprim	16	≤1	2	≤0.5

N/D, not defined.

[a] Breakpoints of dihydrostreptomycin and oxytetracycline were defined microbiologically using 116 isolates of *S. aureus* stored in National Veterinary Assay Laboratory. Breakpoints of remaining antimicrobials were as recommended by the CLSI [12].