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SHORT COMMUNICATION Regional Study

Isolation of methicillin-resistant *Staphylococcus aureus* ST398 from pigs in Japan

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern for public health. Recent decades have seen the emergence and worldwide spread of livestock-associated MRSA, particularly sequence type (ST) 398, in pigs. Two investigations were conducted to confirm the presence of MRSA ST398 in domestic Japanese pigs. In the first investigation, nasal swabs were collected from 500 pigs on 50 pig farms between August 2012 and February 2013. MRSA ST398 was isolated from four pigs from a farm. In the second investigation, nasal swabs were collected from 480 pigs on 24 pig farms between November 2013 and March 2014. MRSA ST398 was isolated from 54 pigs on five farms. These results indicate that MRSA ST398 has become established in domestic Japanese pigs.

Key Words: MRSA, ST398, pig

Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between pigs and humans in European countries was first reported in 2005[2, 26)]. In a Dutch hospital located in a pig-dense area, a three-fold increase in MRSA incidence was observed over a few years[25)]. These reports suggested that pig farming was a risk factor for MRSA carriage in the families of farmers and of their neighbours. Since then numerous studies of MRSA in pigs have been conducted in many countries, showing that some sequence type (ST) clonal lineages of MRSA, such as ST5, ST9, ST97, and ST398 are present in pigs[1, 8, 16, 20, 22)]. These lineages can cause human MRSA infections[2, 6,12, 15, 18)], are called livestock-associated MRSA (LA-MRSA) and are of great concern in human and veterinary medicine. Most LA-MRSA isolates from pigs belong to ST398[8, 16, 22)].

Despite the prevalence of this MRSA ST in other regions, there had been no evidence of it in pigs in Japan. In Japan, approximately one thousand pigs are imported annually[19)], all of which are required to undergo quarantine

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inspections prior to import. However, carriage of MRSA is not inspected because it is not a targeted infectious disease according to the Domestic Animal Infectious Disease Control Law. If pigs infected with MRSA are imported, MRSA could be introduced and spread among domestic pig farms. Evidence of this can be seen in Republic of Korea, which imports breeding pigs from Canada, Denmark and the USA, where MRSA ST398 was isolated from pigs in 2008[16]. According to e-Stat, which is a portal site for the Japanese Government Statistics (https://www.e-stat.go.jp), Japan has imported live pigs from these three countries for breeding purposes. To determine whether MRSA ST398 was present in domestic Japanese pig farms, we conducted two surveys of a large number of pig farms in geographically disparate regions in Japan.

In the first investigation, veterinarians visited a total of 50 pig farms (48 farrow-to-finish and 2 finishing farms) in four regions (ten in Tohoku, 23 in Kanto, seven in Tokai region on Honshu; and ten in Kyushu) between August 2012 and February 2013. These four regions are the main pork producing regions in Japan: accommodating more than 80% of the pigs in the whole country[19]. All farms participated voluntarily on condition of anonymity. On each farm, ten animals (6–29 weeks of age) were selected by veterinarians, totalling 500 pigs. A nasal swab sample was collected from each animal using a BBL CultureSwab Plus Amies Gel with Charcoal, Single Swab (Becton Dickinson, MD, USA). Samples were delivered under refrigeration to the Research Institute for Animal Science in Biochemistry and Toxicology (Kanagawa, Japan) within 24 hr. MRSA isolation was conducted within 48 hr of sampling according to the protocol recommended by the baseline survey on the prevalence of MRSA in holdings with breeding pigs in the EU[24]. In brief, the tip of each nasal swab was added to a tube containing 9 ml of Mueller Hinton broth (Becton Dickinson) containing 6.5% NaCl; the cultures were then incubated for 24 hr at 37 °C. After incubation, 1 ml of the enrichment culture was added to 10 ml tryptic soy broth (Becton Dickinson) containing 3.5 mg/l cefoxitin (Sigma-Aldrich, Tokyo, Japan) and 75 mg/l aztreonam (MP Biomedicals, OH, USA), and incubated for 24 hr at 37 °C. The resulting cultures were grown in CHROMagar MRSA medium (CHROMagar, Paris, France) for 24 hr at 37 °C. When suspected MRSA colonies were observed, up to three colonies per sample were isolated and identified using the API Staph system (Sysmex bioMérieux, Tokyo, Japan). One MRSA isolate per sample was subjected to molecular typing and antimicrobial susceptibility testing. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed using multiplex PCR-based amplification as previously described[13]. MRSA isolates were also characterized by *spa* repeat determination[11] and multilocus sequence typing[9]. Presence of genes encoding Panton-Valentine leukocidin toxin (*pvl*) was determined through PCR amplification[17]. *S. aureus* ATCC BAA-1556 was used as a *pvl*-positive control. Minimal inhibitory concentrations (MICs) of ten antimicrobials (ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, teicoplanin, tetracycline, oxacillin, and vancomycin) were determined using the broth microdilution method in dried plates (Eiken Chemical) following the guidelines of the Clinical and Laboratory Standards Institute[4, 5]. *S. aureus* ATCC 29213 was used for quality control.

MRSA was isolated from five (1%) pigs aged 5 months on two (4%) of the farms surveyed (farms a and d) (Table), both located in the Kanto region. All five MRSA isolates had the class A *mec* gene complex but were negative for *ccr*, and were classified as atypical. The *pvl* gene was not detected in any of these isolates. One MRSA isolate from "Farm a" was classified as *spa* types t002 and ST5 and was found to be resistant to six antimicrobials. The remaining four isolates were obtained from four different pigs reared in the same pen in "Farm d." All isolates were classified as ST398 and a novel *spa* type, t16450 (08-16-02-111-02-25-34-24-25). All four ST398 isolates were resistant to six or more antimicrobials.

Table. Isolation of MRSA from pigs

Farm code	Date of sampling	Prevalence of MRSA	No. of isolates	characteristics of MRSA isolates (ST/*spa* type/*mec* type/SCC*mec* type/antimicrobial resistance profile)
First investigatoin				
a	20 Nov. 2012	1/10	1	ST5/t002/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
d	15 Nov. 2012	4/10	2	ST398/t16450/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC, GM
			2	ST398/t16450/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
Second investigation				
a	28 Jan. 2014	4/20	2	ST5/t002/B/IVb/ABPC, CLDM, CP, EM, MPIPC, TC
			2	ST5/t002/B/IVb/ABPC, CLDM, EM, MPIPC, TC
b	17 Dec. 2013	12/20	1	ST10/t002/B/IVa/ABPC, CLDM, CP, EM, MPIPC, TC
			11	ST10/t002/B/IVa/ABPC, CP, MPIPC, TC
c	4 Feb. 2014	8/20	8	ST97/t1236 /C/V/ABPC, CLDM, CP, EM, MPIPC, TC
d	9 Jan. 2014	18/20	18	ST398/t16450/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
e	9 Jan. 2014	2/20	2	ST398/t16450/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
f	9 Jan. 2014	9/20	6	ST398/t16450/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
			3	ST398/t3934/A/atypical/ABPC, CLDM, CP, EM, MPIPC, TC
g	9 Jan. 2014	11/20	3	ST398/t16450/A/atypical/ABPC, CLDM, CP, CPFX, EM, MPIPC, TC
			8	ST398/t3934/A/atypical/ABPC, CLDM, CP, CPFX, EM, MPIPC, TC
h	12 Mar. 2014	14/20	4	ST398/t034/C/V/ABPC, CLDM, CP, EM, MPIPC, TC
			3	ST398/t034/C/V/ABPC, CLDM, CP, EM, MPIPC
			3	ST398/t034/C/V/ABPC, CLDM, CP, MPIPC, TC
			4	ST398/t034/C/V/ABPC, CLDM, CP, CPFX, EM, MPIPC, TC

Abbreviation; ST: sequence type, ABPC: ampicillin, CLDM, clindamycin, CP: chloramphenicol, CPFX: ciprofloxacin, EM: erythromycin, MPIPC: oxacillin, TC: tetracycline, GM: gentamicin.

In the second investigation, veterinarians visited 24 pig farms (21 farrow-to-finish and 3 finishing farms) in the Kanto region between November 2013 and March 2014. Of the 24 pig farms, 21 had also participated in the first investigation. Nasal swabs were collected from 20 pigs (6–28 weeks of age) from at least two age groups at each farm, with each age group coming from one pen. The pigs tested in both investigations were determined to be healthy based on visual inspections by veterinarians.

MRSA was isolated from 78 pigs on eight farms, all of which had been surveyed in the initial investigation. Of these eight farms, two (farms a and d) were positive for MRSA in the first investigation. MRSA isolates belonging to ST5, ST10, and ST97 were isolated from three farms (a, b, and c). ST5, ST10, and ST97 isolates were classified as *spa* types t002, t002, and t1236, respectively. MRSA belonging to ST398 were isolated from five farms (d, e, f, g, and h) located within a 2 km radius of each other. From a total of 54 MRSA ST398 isolates, 29, 11, and 14 isolates were classified as *spa* types t16450, t3934, and t034, respectively. ST398/t16450, ST398/t034, and ST398/t3934 were isolated from four (d, e, f, and g), two (f and g) and one (h) farms, respectively. All 78 MRSA isolates were negative for *pvl*. SCC*mec* types of ST5/t002, ST10/t002, ST97/t1236, and ST398/t034 were IVb, IVa, V, and V, respectively. SCC*mec* types of ST398/t16450 and ST398/t3934 were classified as atypical, as no amplicons resulted from PCR for *ccr* typing.

Studies on the presence of MRSA in domestic Japanese pigs were conducted at abattoirs in 2009[3] and 2013[23] showed the presence of MRSA ST5, ST97 and ST221, but not MRSA ST398 or ST10. To the best of our knowledge, the isolation of MRSA ST398 from four pigs on a pig farm in the Kanto region in November 2012, is the first isolation of MRSA ST398 in pigs in Japan. Fourteen months later, in January 2014, MRSA ST398 was isolated from pigs on five farms, including the MRSA-positive farm from the first investigation. The number of sampled pigs at each farm in the second survey was twice that of the first. Although the probability of MRSA detection may have increased due to the larger sample size, these findings indicate that MRSA ST398 may have become established in Japan. Three ST398

lineages, t034, t3934 and t16450, were found in the present study. ST398/t034 is one of the major lineages of LA-MRSA in pigs[1, 8, 16,22] and most of which have SCC*mec* type V and are negative for *pvl*[16, 22]. ST398/t3934 is one of the minor lineages in pigs[7]. ST398/t16450 was first isolated in the world. The isolation of MRSA ST10 in pigs has never been reported in the world. High prevalence (60%, 12/20) in pigs in Farm b suggests that ST10 is also adapted to pigs as well as ST5, ST97 and ST398 lineages.

Koyama et al.[14] described the isolation of *pvl*-positive MRSA ST398 from a Chinese woman receiving steroid therapy for systemic lupus erythematosus in a Japanese hospital in 2015, representing the first documented case of MRSA ST398 in a human in Japan, while until 4 days prior to admission to the hospital, the subject had been outside of Japan for approximately 2 months. The patient had had no contact with animals or persons working or living with animals, and the MRSA ST398 isolate obtained was genetically close to a community-associated MRSA lineage detected in China. Thus, the subject was unlikely to have been infected with this isolate from animals in Japan. Nakaminami et al.[21] recently reported a case of intractable arthritis of the shoulder joint caused by *pvl*-positive MRSA ST1232 (a single-locus variant of ST398 belonging to clonal complex 398) in a patient in 2018. The transmission route was unclear because the patient reported no overseas travel or animal contact.

Furuno et al.[10] recently reported that at least 12 of 125 pigs imported from Europe and North America tested positive for MRSA ST398 during the quarantine period. These pigs infected with MRSA ST398 must have been released to domestic pig farms because the import of infected pigs is not prohibited, suggesting that the MRSA ST398 lineages isolated in this study may have been introduced into Japan by imported pigs. In this study, MRSA ST398 was not isolated from any regions other than Kanto. However, 6 years have passed since the first isolation of MRSA ST398. Because repeated importation of pigs infected with MRSA increases the possibility of MRSA spread throughout Japan, it is necessary to monitor the prevalence of MRSA ST398 in pigs throughout Japan.

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Conflict of interest

None to declare.

References

1) Alt K, Fetsch A, Schroeter A, Guerra B, Hammerl JA, Hertwig S, Senkov N, Geinets A, Mueller-Graf C, Braeunig J, Kaesbohrer A, Appel B, Hensel A, Tenhagen BA. Factores associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet Res 7, 69, 2011.
2) Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11, 711-714, 2005.
3) Baba K, Ishihara K, Ozawa M, Tamura Y, Asai T. Isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from swine in Japan. Int J Antimicrob Agents 36, 352- 354, 2010.
4) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. 4rd ed, CLSI document Vet08-4, Wayne, PA: CLSI, 2018.

5) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 28th informational supplement. CLSI document M100-S28, Wayne, PA: CLSI. 2018.
6) Cuny C, Wieler LH, Witte W. Livestock-associated MRSA: the impact on humans. Antibiotics 4, 521-543, 2015.
7) Dierikx CM, Hengeveld PD, Veldman KT, de Hann A, van der Voorde S, Dop PY, Bosch T, van Duijkeren E. Ten years later: still a high prevalence of MRSA in slaughter pigs despite a significant reduction in antimicrobial usage in pigs the Netherlands. J Antimicrob Chemother 71, 2414-2418, 2016.
8) Emborg HD, Porrero MC, Sanders P, Schuepbach G, Teale C, Tenhagen BA, Wagenaar J. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008 - Part A: MRSA prevalence estimates. EFSA Journal 7, 1376, 2009.
9) Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38, 1008-1015, 2000.
10) Furuno M, Uchiyama M, Nakahara Y, Uenoyama K, Fukuhara H, Morino S, Kijima M. A Japanese trial to monitor methicillin-resistant *Staphylococcus aureus* (MRSA) in imported swine during the quarantine period. J Glob Antimicrob Resist 14, 182-184, 2018.
11) Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41, 5442-5428, 2003.
12) Köck R, Schaumburg F, Mellmann A, Köksal M, Jurke A, Becker K, Friedrich AW. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. PLoS One 8, e55040, 2013.
13) Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, HiramatsuK. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec, ccr,* and major differences in junkyard regions. Antimicrob Agents Chemother 51, 264-274, 2007.
14) Koyama H, Sanui M, Saga T, Harada S, Ishii Y, Tateda K, Lefor AK. A fatal infection caused by sequence type 398 methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin gene: A case report in Japan. J Infect Chemother 21, 541 - 543, 2015.
15) Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum M, Skov RL. Pigs as Source of Methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. Emerg Infect Dis 14, 1383-1389, 2008.
16) Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, Kwak HS. The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. Vet Microbiol 155, 88-92, 2012.
17) Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter M, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29, 1128-1132, 1999.
18) Lozano C, Rezusta A, Gómez P, Gómez-Sanz E, Báez N, Martin-Saco G, Zarazaga Myrion, Torres C. High prevalence of *spa* types associated with the clonal lineage CC398 among tetracycline-resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital. J Antimicrob Chemother 67, 330-334, 2012.
19) Ministry of Agriculture, Forestry and Fisheries in Japan. The 89th statistical yearbook of Ministry of Agriculture, Forestry

and Fisheries in Japan, 2015.
20) Molla B, Byrne M, Ableyl M, Mathews J, Jackson CR, Fedorka-Cray P, Sreevatsan S, Wang P, Gebreyes WA. Epidemiology and genotypic characteristics of Methicillin-resistant *Staphylococcus aureus* strains of porcine origin. J Clin Microbiol 50, 3687-3693,2012.
21) Nakaminami H, Hirai Y, Nishimura H, Takadama S, Noguchi N. Arthritis caused by MRSA CC398 in patient without animal contact. Japan. Emerg Infect Dis 26, 795-797, 2020.
22) Normanno G, Dambrosio A, Lorusso V, Samoilis G, Taranto PD, Parisi A. Methicillin-resistant *Staphylococcus aureus* (MRSA) in slaughtered pigs and abattoir workers in Italy. Food Microbiol 51, 51-56, 2015.
23) Sato T, Usui M, MotoyaT, Sugiyama T, Tamura Y. Characterisation of methicillin-resistant *Staphylococcus aureus* ST97 and ST5 isolated from pigs in Japan. J Glob Antimicrob Resist 3, 283-528, 2015.
24) The European Communities. Commission Decision of 20 December 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States (2008/55/EC), 2008.
25) van Rijen MM, Van Keulea PH, Kluytmans JA. Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. Clin Infect Dis 46, 261-263, 2008.
26) Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11, 1965-1966, 2005.