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Effectiveness of ear skin swabs for monitoring methicillin-resistant *Staphylococcus aureus* ST398 in pigs at abattoirs

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ABSTRACT. Monitoring the prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in pigs could be useful for managing transmission risk to humans. To optimize sampling for LA-MRSA monitoring, we compared the sensitivity of MRSA isolation from skin swabs taken behind the ear and nasal swabs collected from 276 pigs and investigated the prevalence of MRSA in their carcasses. MRSA was isolated from 40 behind the ear skin swabs (14.5%), which was statistically higher than the number isolated from nasal swabs (23 samples, 8.3%). MRSA prevalence in the carcasses was 0.4%. All MRSA isolates were sequence type 398 lineage. Sampling of both the skin behind the ear and nasal mucosa in a pig is recommended to investigate the prevalence of LA-MRSA in pigs.

KEY WORDS: methicillin-resistant *Staphylococcus aureus*, pig, sequence type 398

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen that causes healthcare- and community-associated infections in humans. In recent decades, the emergence and increasing prevalence of livestock-associated MRSA (LA-MRSA), particularly sequence type 398 (ST398) belonging to clonal complex 398 (CC398), in pigs have become a global concern. Rearing and slaughtering pigs infected with LA-MRSA and contamination of pork with LA-MRSA are potential risk factors for infection in humans such as farmers, workers at abattoirs, and pork consumers [13, 15, 19–22]. In Japan, ST398 MRSA was first isolated from pigs on a farm in Kanto region in 2012, and 1 year later, it was observed in pigs on seven farms [16]. We isolated ST398 MRSA from pigs slaughtered at two abattoirs in Tohoku region between May and October 2017, and the infected pigs were shipped from nine farms in Tohoku region [17]. The results of these studies suggest that LA-MRSA has already disseminated on pig farms in Japan. Therefore, it is critical to monitor the prevalence of LA-MRSA in pigs throughout the country and characterize the isolates. In previous Japanese studies, nasal swabs were used for the isolation of MRSA from pigs. However, Agersø *et al.* [1] reported that swabs of the skin behind the ear are more sensitive for MRSA isolation in pigs than nasal swabs. In addition, although the prevalence of LA-MRSA in pork is needed to estimate the risk of its transmission from contaminated pork to humans, there have been no reports on the isolation of LA-MRSA from the carcasses of pigs in Japan. This study aimed to compare the sensitivity of MRSA isolation using swabs of the skin behind the ear and nasal swabs and to investigate the prevalence of MRSA in the carcasses of pigs slaughtered at abattoirs.

Sampling was conducted at the three abattoirs investigated in our previous study [17]. Swabs of the skin (25 cm^2) behind the

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ear, nasal mucosa, and edible part (neck, 25 cm^2) of the carcass were collected from 276 pigs (three per farm) shipped from 92 farms using cotton swabs (SEEDSWAB No. 1; Eiken Chemical, Tokyo, Japan) in June and July 2019. The 92 farms were located in four prefectures of the Tohoku region. All pigs were diagnosed as healthy upon visual inspection by veterinarians. The collected samples were delivered under refrigeration to the Towada Meat Inspection Center (Aomori, Japan).

At the center, MRSA isolation was conducted within 6 hr after sampling. The tip of each swab was inoculated into 9 ml of Mueller Hinton (MH) broth (Kanto Chemical, Tokyo, Japan) containing 6.5% NaCl and incubated for 18–24 hr at 37°C, for enrichment. After incubation, a loopful of the MH culture was plated onto CHROMagar™ MRSA medium (CHROMagar, Paris, France) and Pourmedia® MRSA II medium (Eiken Chemical) and incubated for 48 hr at 37°C. When suspected MRSA colonies were observed on these media, up to two colonies per sample were picked up and identified using commercial identification kit (CycleavePCR™ *Staphylococcus aureus* [DnaJ gene] Detection Kit; Takara Bio, Kusatsu, Japan). One MRSA isolate per sample was subjected to molecular typing and antimicrobial susceptibility testing. MRSA isolates were characterized by multilocus sequence typing (MLST) [5] and *spa* typing [8]. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was also performed by multiplex PCR amplification of the *mec* and *ccr* regions using the primers described by Kondo *et al.* [10]. The isolates were tested for the presence of *czrC*, *pvl* and *hlg* genes encoding cadmium and zinc resistance, Panton-Valentine leukocidin and γ-hemolysin, respectively, by PCR, as previously described [2, 12]. *S. aureus* ATCC strain BAA-1556 was used as a control in the identification procedures. MRSA isolates were also tested for susceptibility to ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, trimethoprim, gentamicin, teicoplanin, tetracycline, oxacillin, and vancomycin. The minimal inhibitory concentrations (MICs) of these antimicrobials were determined using the broth microdilution method on dried plates (Eiken Chemical) according to Clinical and Laboratory Standards Institute guidelines [3, 4]. *S. aureus* ATCC 29213 was used for quality control.

MRSA was isolated from 48 (17.4%) pigs shipped from 25 (27.2%) farms (Table 1). Of the 48 MRSA-positive pigs, 25 and 8 had MRSA on the skin behind the ear or the nasal mucosa only, respectively; 14 pigs had MRSA on both the skin behind the ear

Table 1. Isolation sites and characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in MRSA-positive pigs

Abattoir code	Farm code	Prefecture code	No. of positive pigs	Isolation sites				No. of isolates	Characteristics of MRSA isolates (ST/ *spa* type/ *SCCmec* type/ antimicrobial resistance profile)
				SE	NA	SE+NA	SE+NA+NC		
aa	fa	pa	3	2	0	1	0	4	ST398/ t034/ V/ ABPC, TC, TMP
	fb	pa	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fc	pb	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fd	pa	2	2	0	0	0	2	ST398/ t034/ V/ ABPC, TC, TMP
	fe	pa	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	ff	pa	3	2	0	1	0	4	ST398/ t034/ V/ ABPC, TC, TMP
	fg	pa	1	1	0	0	0	1	ST398/ t19022/ V/ ABPC, TC, TMP
	fh	pc	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fi	pb	3	3	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
								2	ST398/ t19022/ V/ ABPC, TC, TMP
	fj	pb	2	2	0	0	0	2	ST398/ t034/ V/ ABPC, TC, TMP
	fk	pa	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fl	pa	3	1	0	2	0	5	ST398/ t034/ V/ ABPC, TC, TMP
ab	fm	pa	3	0	1	2	0	1	ST398/ t034/ V/ ABPC, TC, TMP
								4	ST398/ t034/ V/ ABPC, TC, CP, TMP
	fn	pa	2	0	1	1	0	3	ST398/ t034/ V/ ABPC, TC, TMP
	fo	pa	1	0	1	0	0	1	ST398/ t034/ IVa/ ABPC, TC
	fp	pc	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fq	pa	2	1	1	0	0	2	ST398/ t034/ V/ ABPC, TC, TMP
	fr	pb	1	0	0	0	1	2	ST398/ t034/ V/ ABPC, TC, TMP
								1	ST398/ t034/ V/ ABPC, TC, CP, TMP
	fs	pb	3	0	0	3	0	3	ST398/ t034/ V/ ABPC, TC, TMP
								3	ST398/ t034/ V/ ABPC, TC, CP, TMP
	ft	pb	1	1	0	0	0	1	ST398/ t034/ V/ ABPC, TC, TMP
	fu	pb	3	1	0	2	0	5	ST398/ t034/ V/ ABPC, TC, TMP
ac	fv	pa	3	1	2	0	0	2	ST398/ t034/ V/ ABPC, TC, TMP
								1	ST398/ t034/ V/ ABPC, TC, CP, TMP
	fw	pa	2	1	1	0	0	2	ST398/ t1928/ V/ ABPC, TC
	fx	pb	1	1	0	0	0	1	ST398/ t034/ IVa/ABPC, TC, CP, TMP
	fy	pb	3	0	1	2	0	5	ST398/ t034/ V/ ABPC, TC, TMP
	Total		48	25	8	14	1	64	

SE: skin behind the ear, NA: nasal mucosa, NC: neck of the carcass, ST: sequence type, SCC*mec*: staphylococcal cassette chromosome *mec*, ABPC: ampicillin, TC: tetracycline, CP: chloramphenicol, TMP: trimethoprim.

and the nasal mucosa; and the remaining pig had MRSA on all three sampling sites. The sampling site with the highest prevalence was the skin behind the ear (14.5%, 40/276), and the prevalence at this site was statistically higher (Fisher's exact test; $P<0.05$) than that in the nasal mucosa (8.3%, 23/276). Sampling both the skin behind the ear and the nasal mucosa of a pig was the most sensitive for MRSA isolation. However, no significant difference (Fisher's exact test; $P>0.05$) was found in the isolation rate between combination sampling (17.4%, 48/276) and sampling just the skin behind the ear (14.5%, 40/276).

The 64 isolates from the 48 MRSA-positive pigs were first subjected to molecular typing, and all isolates were ST398 (allelic profile 3-35-19-2-20-26-39). Of the 64 MRSA ST398 isolates, 59, 2, and 3 were classified as *spa* types t034, t1928, and t19022, respectively. The *SCCmec* types of 57 t034 isolates from pigs from 21 farms and two t034 isolates from pigs from two farms were V and IVa, respectively. The two t1928 isolates were isolated from two pigs on Farm fw, and these isolates were *SCCmec* type V. The *spa* type t19022 was a novel type (08-16-02-25-02-25-34-25-51), and the three t19022 isolates were obtained from two farms. All three t19022 isolates were *SCCmec* type V. All 62 isolates carrying type V *SCCmec* elements were positive for *czrC*, whereas the two isolates carrying type IVa *SCCmec* elements were negative for *czrC*. All the ST398 isolates were positive for the γ-hemolysin gene but negative for *pvl*.

Next, the 64 isolates were subjected to antimicrobial susceptibility testing. All isolates were resistant to ampicillin, oxacillin, and tetracycline but were susceptible to cefazolin, gentamicin, kanamycin, ciprofloxacin, levofloxacin, colistin, minomycin, erythromycin, clindamycin, vancomycin, and teicoplanin. Most (61 95.3%) isolates were resistant to trimethoprim; however, one ST398/t034/IVa isolate from a pig on farm fo and two ST398/t1928/V isolates from two pigs on farm fw were susceptible to trimethoprim. Of the isolates on a pig carrying ST398/t034 strains at all sampling sites, both isolates from the skin behind the ear and the neck of the carcass were susceptible to chloramphenicol, whereas the other isolate from the nasal mucosa was resistant to chloramphenicol.

An ST398 MRSA strain was isolated from the neck of the carcass of a pig in this study, which is the first-ever report of an ST398 MRSA isolate from a pig carcass in Japan. In this study, we collected swabs of the neck because bacterial contamination levels in the neck are higher than in other edible parts of pig carcass [18, 23]. Although the contamination rate was very low (0.4%, 1/276), it could increase. There have been only two case reports of CC398 MRSA isolation from patients in Japan. In one case, the patient was diagnosed with systemic lupus erythematosus and had stayed in China for approximately 2 months before admission to a Japanese hospital, and the ST398 isolate carried a *pvl* gene and was genetically close to a community-associated MRSA lineage isolated in China [11]. In the other case, the patient had intractable arthritis of the shoulder joint and had reported no overseas travel or animal contact, this MRSA isolate was classified as ST1232 (a single-locus variant of ST398) and was positive for the *pvl* gene [14]. There have been no reports of *pvl*-positive CC398 MRSA isolates from pigs in Japan. Thus, these two patients were unlikely to have been infected with CC398 by eating domestic pork or through contact with pigs in Japan.

The results of our current and previous studies [17] showed that ST398 MRSA has spread on pig farms in the Tohoku region. Pigs used for breeding in Japan are imported from Europe and North America, where ST398 MRSA is prevalent [6, 9, 22], and imported pigs and their offspring are transferred to pig farms throughout Japan. Furuno *et al.* [7] investigated the presence of MRSA in 125 pigs belonging to 15 lots (unit of import) imported from these areas from July 2016 to February 2017, and isolated ST398 MRSA from six (40%) lots, indicating that ST398 MRSA must have spread to regions other than Tohoku. Therefore, it is necessary to monitor the prevalence of LA-MRSA in pigs throughout the country and genotypically and phenotypically characterize the isolates. Sampling both the skin behind the ear and the nasal mucosa of pigs should be recommended to investigate the prevalence of MRSA in pigs. However, considering the high cost and labor required for sampling, the skin behind the ear could be a scientific, rational sampling site option.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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