Sequence type 72 methicillin-resistant *Staphylococcus aureus* isolates from humans, raw meat and soil in South Korea

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The characteristics and relationships of sequence type (ST) 72 *Staphylococcus aureus* isolates from humans, raw meat and soil in South Korea were investigated. Several close relationships based on molecular evidence such as staphylococcal cassette chromosome *mec* (SCC*mec*) type, *spa* gene type, PFGE, the presence of virulence genes and the nucleotide sequences of 12 chromosomal genes suggested the transmissibility of ST72 methicillin-resistant *S. aureus* (MRSA) isolates among humans, livestock and the environment. In addition, the results indicated that ST72 MRSA may have originated from ST72 methicillin-susceptible *S. aureus*, but the acquisition of SCC*mec* has not occurred repeatedly.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen that causes surgical site infections, pneumonia and bloodstream infections in hospitalized patients. Since the late 1990s, however, MRSA has been rapidly emerging in communities with no MRSA risk factors. Community-associated MRSA (CA-MRSA) is now established in many countries including South Korea (Kim *et al.*, 2007).

In the USA, the most representative CA-MRSA clone is USA300, which is sequence type (ST) 8 and staphylococcal cassette chromosome *mec* (SCC*mec*) type IV (King *et al.*, 2006; Moran *et al.*, 2006). However, several studies have reported that the major CA-MRSA clone in South Korea is ST72 (Kim *et al.*, 2007; Ko *et al.*, 2008; Park *et al.*, 2009). Although ST72 MRSA in Korea possesses SCC*mec* type IVA, it lacks the Pantón–Valentine leukocidin (*pvl*) gene, unlike the USA300 clone. Recently, ST72 MRSA isolates were also found in raw meat in South Korea (Lim *et al.*, 2010). In this study, we compared ST72 MRSA isolates from humans, raw meat and soil in South Korea.

METHODS

A total of 12 ST72 *S. aureus* isolates from South Korea were analysed (Table 1). Four ST72 MRSA isolates from hospitalized patients were selected randomly from 84 isolates collected in four surveillance studies, which were carried out by the Asia Pacific Foundation for Infectious Diseases in South Korea. Three ST72 methicillin-susceptible *S. aureus* (MSSA) isolates from humans were selected from five ST72 MSSA isolates collected in the same studies. Four MRSA isolates were from beef and pork (Lim *et al.*, 2010), and one MRSA strain was isolated from soil unrelated to a slaughterhouse. Antimicrobial susceptibility testing was performed using a broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2009). Multilocus sequence typing, SCC*mec* typing, *spa* typing and PFGE were performed as described previously elsewhere (Enright *et al.*, 2000; Koren *et al.*, 2004; Milheirico *et al.*, 2007; Mulvey *et al.*, 2001; Oliveira & de Lencastre, 2002). Enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sek* and *tst*) and the *pvl* gene were also screened using PCR methods (Jarraud *et al.*, 2002). To compare the genetic backgrounds of the ST72 *S. aureus* isolates, the nucleotide sequences of 12 chromosomal regions, all of which flank essential housekeeping genes, were determined: SA004 (*recF*), SA124 (a gene encoding a hypothetical protein similar to glycosyltransferase TaaA), SA342 (*yqiL*), SA560 (a gene encoding a hypothetical protein), SA729 (*tpi*), SA1052 (*gmk*), SA1244 (*odhB*), SA1792 (a gene encoding a ssDNA-binding protein), SA2020 (a gene encoding a hypothetical protein similar to the ABC transporter), SA2414 (a gene encoding a hypothetical protein similar to glutathione peroxidase), SA2425 (*arcC*) and SA2499 (*gidB*). These genes were identified as essential genes for growth in our previous knockout study (Ko *et al.*, 2006).

Abbreviations: CA, community-associated; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S. aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; ST, sequence type.

Table 1. List and characteristics of ST72 MRSA and MSSA isolates investigated in this study

The *pvl* gene was not detected in any of the isolates.

Origin	No. of isolate	Year	<i>spa</i> type*	SCCmec†	MIC (mg l ⁻¹)‡					
					OXA	ERY	CLI	TET	SXT	VAN
Human	K01-140	2006	t324 (UJGGMDMGGM)	IVA	64	0.25	0.12	0.12	0.12/2.37	0.5
	K07-016	2006	t148 (UJGFGMDMGGM)	IVA	64	0.25	0.25	≤0.06	0.06/1.18	0.5
	K07-204	2007	t664 (UJGGMDMGGM)	IVA	64	>64	0.25	<0.06	0.12/2.36	0.5
	K07-322	2007	t664 (UJGGMDMGGM)	IVA	64	32	0.25	<0.06	0.06/1.18	0.5
	K01-799	2006	t2703 (UJGFMGM)	NA	0.5	0.25	0.12	0.25	0.06/1.18	0.5
	K07-006	2006	t126 (UJGFMGM)	NA	0.5	0.25	0.12	0.25	0.06/1.18	0.5
	K07-561	2007	t126 (UJGFMGM)	NA	0.25	0.5	0.25	<0.06	<0.03/0.59	0.5
Animal	05-B-52	2005	t324 (UJGGMDMGGM)	IVA	32	64	0.12	0.5	<0.25	1
	05-B-60	2005	t1346 (UJGFGMDMGGGM)	IVA	16	2	0.12	0.5	<0.25	1
	08-B-93	2008	t324 (UJGGMDMGGM)	IVA	32	2	0.12	0.5	<0.25	1
	08-P-236	2008	Unknown (UJGGGDGGM)	IVA	16	64	0.25	0.5	<0.25	1
Soil	4-009	2009	t324 (UJGGMDMGGM)	IVA	16	>64	0.25	0.125	0.0625	0.5

*Letters in parentheses represent individual repeats.

†NA, Not available (MSSA).

‡OXA, Oxacillin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

RESULTS

All 84 ST72 MRSA isolates from humans were SCCmec type IVA, a general feature of CA-MRSA. The other MRSA isolates from raw meats and soil were also SCCmec type IVA. The 84 ST72 MRSA isolates from humans and the other MRSA isolates from raw meats and soil did not show multidrug resistance; all isolates showed susceptibility to clindamycin, tetracycline and trimethoprim-sulfamethoxazole, another feature typical of CA-MRSA isolated in South Korea (Kim *et al.*, 2007; Ko *et al.*, 2008; Park *et al.*, 2009). Analysis of enterotoxin genes found that the 12 *S. aureus* isolates explored in this study harboured the *seg*, *seh*, *sei* and *sek* genes.

There were seven *spa* types within the 12 ST72 *S. aureus* isolates, as shown in Table 1. These types were similar to each other and belonged to a single *spa* clonal complex (*spa*-CC324). The PFGE results of the 12 *S. aureus* isolates also showed similar patterns to one other, supporting the suggestion of a common ancestor (Fig. 1). The results of *spa* typing and PFGE were consistent: four MRSA isolates possessing *spa* type t324 (K01-140, 05-B-52, 08-B-93 and 4-009) showed the same PFGE patterns, along with two MRSA isolates with *spa* type t664 (K07-204 and K07-322) and a new *spa* type (08-P-236). Notably, the four MRSA isolates showing the same *spa* type and PFGE pattern originated from humans, raw meats or soil. In contrast, the three MSSA isolates from humans showed different PFGE patterns from those of the MRSA isolates (Fig. 1). However, the difference of just one band between the MRSA and MSSA isolates may have been due to the presence or absence of the SCCmec element.

We determined the nucleotide sequences of 15 986 bp from 12 chromosomal regions, and 76 variable sites were

obtained. Based on these nucleotide sequences, the phylogenetic tree of the ST72 *S. aureus* isolates was reconstructed (Fig. 2). The phylogenetic tree showed three distinct clusters, supported by bootstrap analysis. Two clusters were homogeneous: the first was composed of two MRSA isolates from animals (05-B-52 and 05-B-60) and the second consisted of the three MSSA isolates (K01-799, K07-006 and K07-561). However, the third cluster was heterogeneous, including four MRSA isolates from humans (K01-140, K07-016, K07-204 and K07-322), two MRSA isolates from raw meat (08-B-93 and 08-P-236) and the MRSA isolate from soil (4-009). Thus, two MRSA isolates from raw meat clustered with MRSA isolates from humans and soil, but the other two MRSA isolates from raw meat formed an independent and divergent clade. The two MRSA isolates forming the divergent clade (05-B-52 and 05-B-60) co-diverged from other isolates in two loci, SA004, encoding *recF* and its flanking regions, and SA1792, encoding a ssDNA-binding protein. In addition, the three ST72 MSSA isolates co-diverged from MRSA isolates in SA1792.

DISCUSSION

To date, ST72 MRSA isolates have rarely been reported in countries other than South Korea. Those ST72 MRSA isolates reported in other countries were sporadic and not disseminated (Ghebremedhin *et al.*, 2009; Schuenck *et al.*, 2009). Thus, we assume that ST72-MRSA-IVA is an MRSA clone specific to South Korea. The relationships among ST72 *S. aureus* isolates, the representative clone of CA-MRSA in South Korea, was investigated. It has been suggested that ST72 MRSA isolates first emerged in the community and disseminated into hospitals (Park *et al.*, 2009). Now, ST72

Fig. 1. PFGE results of the 12 ST72 *S. aureus* isolates. PFGE was performed as described previously (Mulvey *et al.*, 2001). Agarose plugs containing genomic DNA were digested with *Sma*I. Electrophoresis was performed with a CHEF Mapper apparatus (Bio-Rad Laboratories) at 6 V cm⁻¹ for 22 h.

MRSA isolates are frequently found as agents of nosocomial infections in South Korea (Bae *et al.*, 2010). No ST72 *S. aureus* isolates included in this study possessed the *pvl* gene, in contrast to CA-MRSA from the USA (USA300 clone). This absence of the *pvl* gene in CA-MRSA isolates from South Korea has been reported repeatedly (Kim *et al.*, 2007; Ko *et al.*, 2008; Park *et al.*, 2009).

It has been reported that the main clone of livestock-associated MRSA in Europe and North America is ST398.

These ST398 MRSA isolates are of concern due to their transmissibility to humans (Golding *et al.*, 2010). Although ST398 MRSA has not been reported so far in South Korea, ST72 MRSA has emerged as the main clone of raw meat-associated MRSA in South Korea (Lim *et al.*, 2010). In the current study, ST72 *S. aureus* isolates, both MRSA and MSSA, showed very homogeneous features such as SCCmec type, *spa* type, distribution of virulence genes and PFGE types, which may indicate that these ST72 *S. aureus* isolates originated from a common ancestor. However, the

Fig. 2. Neighbour-joining tree of the 12 ST72 *S. aureus* isolates based on nucleotide sequences of 12 dispersed chromosomal loci. This midpoint-rooting tree was constructed using PAUP. Numbers at branching nodes are percentages of 1000 bootstrap replications. Only values >50% are shown.

nucleotide sequences of 12 chromosomal regions represented detectable divergence. Thus, the ST72 MRSA isolates from humans were closely related to some ST72 MRSA isolates from raw meat. In addition, an MRSA isolate from soil also showed a close relatedness to these other strains. In contrast, three ST72 MSSA isolates formed a group distinct from the MRSA isolates.

Our study also suggested that ST72 MRSA has not emerged repeatedly from ST72 MSSA. Judging from the clade of ST72 MSSA isolates distinct from the MRSA isolates, ST72 MRSA might have originated from ST72 MSSA, but the uptake of SCCmec type IVA is not recent and did not occur repeatedly. Further investigation including more ST72 MRSA and MSSA isolates could unveil the detailed evolution of ST72 MRSA in South Korea, although ST72 MSSA isolates have rarely been found in both hospital and community settings (Ko *et al.*, 2008).

In summary, we showed that ST72 MRSA isolates from humans, raw meat and soil shared the same characteristics. Some ST72 MRSA isolates from meat and soil showed close relationships with ST72 MRSA isolates from humans, suggesting the possibility of transmission of ST72 MRSA among humans, animals and the environment.

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