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Molecular epidemiology of community-associated and hospital-associated methicillin-resistant *Staphylococcus aureus* in a Japanese university hospital

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Abstract

◎Introduction:
Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has been reported in healthcare facilities worldwide. But details of CA-MRSA in Japanese healthcare facilities are rarely reported. The aim of this study is to know the distribution of CA-MRSA and healthcare-associated MRSA (HA-MRSA), and detailed molecular epidemiology in a Japanese hospital.
◎Methods:
From July to October in 2015, first MRSA isolate from each patient was collected in Juntendo University Hospital, Tokyo, Japan. MRSA strains were categorized as CA-MRSA and HA-MRSA according to the clinical definition of CDC. Detection of toxin genes and SCC*mec* typing were performed by PCR. Genetic relatedness among isolates was determined by pulsed-field gel electrophoresis (PFGE). Multilocus sequence typing was performed using whole genome sequencing data.
◎Result:
A total of 102 MRSA strains were collected in the study period, and categorized as 34 CA-MRSA (33.3 %) and 68 HA-MRSA (66.7 %), respectively. Among the 73 strains isolated from hospitalized patients, 10 were CA-MRSA (13.7 %). Whereas among the 29 strains isolated in clinic, 5 were HA-MRSA (17.2 %). Three major types were as follows: ST8-SCC*mec* IV (n= 26, 25.5 %, CA: HA= 10: 16), ST5-SCC*mec* IIa (n= 17, 16.7 %, CA: HA= 5: 12), and ST1-SCC*mec* IVa (n= 13, 12.7 %, CA: HA= 6: 7). Among ST8-SCC*mec* IV strains, SCC*mec* IVl, originally reported in Japanese CA-MRSA, was found both in CA-MRSA and HA-MRSA (n= 11, 10.8 %, CA: HA= 5: 6). Only one ST772-SCC*mec* V strain carried Panton-Valentine leukocidin (PVL) gene. Two ST764-SCC*mec* IIa strains in ICU, 2 ST764-SCC*mec* IIa strains in a general ward, and 6 ST2764-SCC*mec* IVa strains in NICU showed genetic relatedness by PFGE, respectively. Especially, ST2764-SCC*mec* IVa was a clone originally reported as HA-MRSA in another Juntendo-affiliated hospital.
◎Conclusion:
CA-MRSA and HA-MRSA were comparably found both in hospital and clinic. Unique Japanese clones were found in this study, but it seemed impossible to distinguish CA-MRSA and HA-MRSA simply by ST-SCC*mec* typing. This heterogenous population structure of MRSA suggested that conventional HA-MRSA had lost its predominance by sufficient infection control, resulting in relative increase of CA-MRSA in hospital environment.

Background

◎MRSA has been a most important pathogen causing healthcare-associated infection since firstly reported in 1961.
◎Since 1990s, community-associated MRSA(CA-MRSA) has reported all around the world.
◎In Japan, predominant clone of hospital-associated MRSA(HA-MRSA) is NewYork/Japan clone(ST5/SCC*mec*IIa).
◎CA-MRSA has diversity genetically in Japan. ST8/SCC*mec*IVl, an original Japanese clone, appeared from 2000s.
◎Genetic structure of HA- and CA-MRSA in Japanese hospitals is not known clearly.

Method

◎Study duration:
July to October in 2015
◎Setting:
Juntendo University Hospital, Tokyo, Japan.
(1,026 beds tertiary care teaching hospital)
◎Subject:
First MRSA isolate from each patient
◎MRSA categorize definition:
Clinical definition of CDC.

◎Antimicrobial susceptibility :
Broth microdilution method was used according to the guideline of the Clinical Laboratory Standard Institute (CLSI).
Eiken Dry plate DP32 (Eiken Chemical Co, Tokyo, Japan) was used for minimum inhibitory concentration (MIC) determination.
◎PFGE:
Pulsed-field gel electrophoresis with *SmaI* digestion was performed. Reference strains were N315 and USA300.
BioNumerics software 7.5 (Applied Maths, Saint-Martens-Latem, Belgium) was used for cluster analysis.
◎Toxin gene, SCC*mec* Typing:
Toxin gene detection and SCC*mec* typing were performed by multiplex PCR as reported previously (Becker K et al. JCM 1998, Monday SR et al. JCM 1999, Ma XX et al. JCM 2008, Kondo Y et al. AAC 2007).
SCC*mec*IVl subtyping was performed in accordance with protocol reported previously (Hosoya S et al. Kansenshogaku Zasshi. 2014).
◎MLST:
All strains were implemented to whole genome sequencing by MiSeq (illumina, San Diego, CA, USA).
For multi-locus sequence typing (MLST), assembled genome reads were analyzed by using Center for Genomic Epidemiology website.
(http://www.genomicepidemiology.org).

◎Ethical approval was obtained from the Institutional Review Board of Juntendo University School of Medicine with approval number 15-138.

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Result

Table 1. Distribution of 102 MRSA at Juntendo Hospital.

		Hospitalized patient		Clinic patient		Total
		CA-MRSA n=10	HA-MRSA n=63	CA-MRSA n=24	HA-MRSA n=5	n=102
Age(minimum, median, maximum)		0, 75.5, 94	0, 64, 94	2, 63.5, 89	4, 64, 79	0, 65, 94
Gender(male)		9(90.0%)	39(61.9%)	12(50.0%)	2(40.0%)	62(60.8%))
SCC*mec*	I	0	3	0	0	3
	II	3	23	6	2	34
	IV	5	35	17	3	60
	V	1	1	1	0	3
	NT	1	1	0	0	2
ST	1	2	6	4	1	13
	5	2	17	5	1	25
	8	3	19	7	1	30
	764	1	8	2	1	12
	2764	0	6	0	0	6
	others(#)	1	7	6	1	15
	NT	1	0	0	0	1
Total		10	63	24	5	102

: "others" includes ST81, ST97, ST121, ST188, ST512, ST772, ST834, ST870, ST2725
NT: non-typable

Table 2. Genetic feature of the predominant clones.

		ST5 / SCC*mec*IIa n=17	ST1 / SCC*mec*IVa n=13	ST764 / SCC*mec*IIa n=12	ST8 / SCC*mec*IVl n=11
Hospitalized patient : Clinic patient	HA : CA	12 : 5	7 :6	9 : 3	6 : 5
		12 : 5	8 : 5	9 : 3	8 : 3
Resistance	GM	12 (70.6%)	3 (23.1%)	11 (91.7%)	10 (90.9%)
	EM	17 (100.0%)	12 (92.3%)	12 (100.0%)	2 (18.2%)
	CLDM	17 (100.0%)	12 (92.3%)	12 (100.0%)	2 (18.2%)
	LZD	5 (29.4%)	1 (7.7%)	5 (41.7%)	1 (9.1%)
	ST	4 (23.5%)	0 (0.0%)	1 (8.3%)	0 (0.0%)
	LVFX	17 (100.0%)	13 (100.0%)	12 (100.0%)	2 (18.2%)
Toxin gene	*eta*	3 (17.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	etb	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	tst	11 (64.7%)	0 (0.0%)	2 (16.7%)	9 (81.8%)
	sea	0 (0.0%)	11 (84.6%)	0 (0.0%)	0 (0.0%)
	seb	0 (0.0%)	0 (0.0%)	6 (50.0%)	0 (0.0%)
	sec	13 (76.5%)	0 (0.0%)	2 (16.7%)	9 (81.8%)
	seg	17 (100.0%)	0 (0.0%)	12 (100.0%)	0 (0.0%)
	seh	0 (0.0%)	13 (100.0%)	0 (0.0%)	0 (0.0%)
	sei	17 (100.0%)	0 (0.0%)	12 (100.0%)	0 (0.0%)

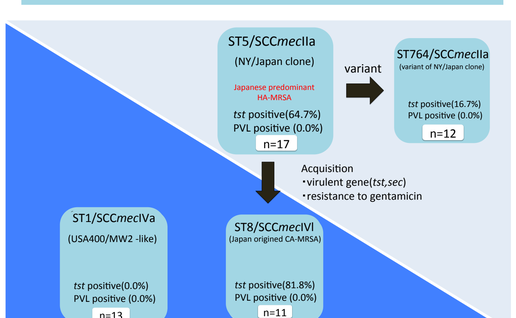

Figure 1. Genetic association between predominant clones at Juntendo Hospital.

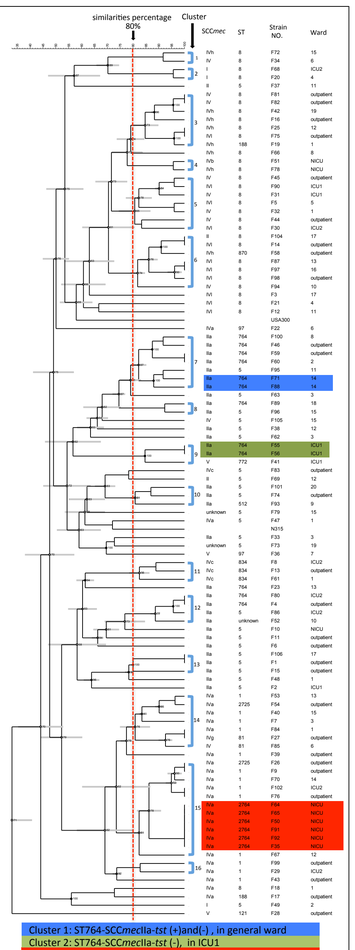

Figure 2. PFGE dendrogram of all strains

Summary of Result

・Ratio of HA and CA-MRSA among both of hospitalized patients and clinic patients were similar.

・Both of HA-MRSA and CA-MRSA included variety of clones.

・In Juntendo Hospital, the most prevalent MRSA clone was NY/Japan Clone (ST5/SCC*mec*IIa), as Japanese conventional HA-MRSA, followed by ST1/SCC*mec*IVa (USA400/MW2 clone), ST764/SCC*mec*IIa (variant of NY/Japan clone), and ST8/SCC*mec*IVl (Japanese original CA-MRSA).

・Only one ST772-SCC*mec* V strain carried Panton-Valentine leukocidin gene.

・ST1/SCC*mec*IVa and ST8/SCC*mec*IVl, which had been reported as CA-MRSA clone, kept sensitivity to multiple antibacterial agents. However, ST8/SCC*mec*IVl possessed *tst* in higher percentage than NY/Japan Clone.

・Outbreak of particular strains was not found although small horizontal transmissions happened in 3 different sections.

Discussion

・Detection of CA-MRSA in Japanese hospitals have been increased (1). Present study offers similar tendency.

・PVL positive strains were rare in Japan consistently with previous reports (2).

・Though ST8-SCC*mec*IVl exhibited high similarity with USA300 (3), possession of *tst* in high frequency is unique. ST8-SCC*mec*IVl disseminated not only community but also hospitals.

・Infection control seemed to be effective to prevent outbreak throughout the hospital. This effect also influenced to decrease NY/Japan clone in hospitals.

・Since its clonal diversity, MRSA can't be classified by a single molecular epidemiologic typing method. Infection control practitioner needs caution at making decision of horizontal transmission, based on the result of molecular typing.

Conclusion

・CA-MRSA and HA-MRSA were comparably found both in hospital and clinic.

・Unique Japanese clones were found in this study, but it seemed impossible to distinguish CA-MRSA and HA-MRSA simply by ST-SCC*mec* typing.

・This heterogenous population structure of MRSA suggested that NY/Japan clone, conventional Japanese HA-MRSA, had lost its predominance by sufficient infection control, resulting in relative increase of CA-MRSA in hospital environment.

Reference

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