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Multilocus Sequence Typing of Clonal Changes of Methicillin-resistant *Staphylococcus aureus* Isolated from Intensive Care Unit Patients: 1996 versus 2004

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배 경 : 한국의 3차 진료기관에서 분리되는 황색포도구균의 70% 이상은 methicillin 내성으로 보고된 바 있다. 최근 multilocus sequence typing (MLST) 기법은 환자에서 분리되는 methicillin-resistant *Staphylococcus aureus* (MRSA)에 대해 지역 및 국가간의 역학분석을 위해 흔히 사용되고 있다. 저자들은 동일한 병동에서 일정한 기간을 두고 분리된 MRSA 균주들을 대상으로 유전적 배경을 비교해보고자 하였다.

방 법 : 한국의 1개 3차 진료기관의 중환자실에서 분리된 MRSA를 대상으로 하였다. 1996년에 분리된 16주, 2004년에 분리된 17주를 수집하여 8년간 중환자실에서 분리된 MRSA의 균형의 변화를 알아보고자 하였다. 디스크 확산법으로 항균제 감수성 검사를 시행하였고, MLST를 시행해 각각의 sequence type을 결정하였다.

결 과 : 1996년 분리주 중 ST5(n=11, 68.7%)가 가장 흔한 형이었고, ST254 (n=3, 18.8%), ST1(n=2, 12.5%)의 형들이 분리되었다. 2004년에는 ST5은 6균주에서만 확인되었고 ST239(n=10, 58.8%)로 가장 흔한 형이 바뀌었다.

결 론 : 중환자실에서 분리된 MRSA의 sequence type은 1996년에는 ST5가 가장 우세하였으나, 2004년에는 ST239로 변화하였다.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections and is also being recovered at an increasing rate in the community. MRSA is usually resistant to all β-lactams and to a wide range of other antibiotics[1]. Their susceptibilities are frequently limited to glycopeptides and a few recently developed drugs. Moreover, recently, MRSA was found to have developed resistance to vancomycin. In Korea, the frequency of methicillin resistance among staphylococcal isolates has increased over the last decade, and in 2003, MRSA was reported to account for more than 70% of the *S. aureus* isolates examined at Korean tertiary-care hospitals[2].

Several molecular techniques, such as pulsed field gel electrophoresis (PFGE), plasmid profile analysis, and random amplified polymorphic DNA (RAPD) analysis, have been used to investigate the epidemiology of MRSA. Recently, a multilocus sequence typing (MLST) scheme has been used to facilitate studies on the local and global epidemiologies of MRSA[3,4]. The aim of this study was to compare the genetic background of MRSA strains isolated in the same ward during two different periods. In the present study, we utilized MLST to examine MRSA isolates collected in 1996 and 2004 from intensive care units (ICUs) at Seoul National University Hospital, Seoul, Korea.

MATERIALS AND METHODS

A total of 33 MRSA isolates was collected from patients hospitalized in these ICUs. Six cases of MRSA bacteremia occurred in the ICUs in 1996, and 23 cases in 2004. The strains collected included two MRSA categories: (1) 16 MRSA strains isolated in 1996 (6 blood and 10 other clinical

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Table 1. Characteristics of patients with MRSA infection

No. of isolates	Age (years)	Gender	Services	Department	Source of specimen	Diagnosis	Outcome	Date of isolation (mm-dd-yy)
1996								
96-2	46	F	Neurosurgery	SICU	Blood	Herniated nucleus pulposus	Survived	05/25/96
96-3	54	M	Neurosurgery	SICU	Bed sore	Brain tumor	Expired	06/11/96
96-4	41	F	Neurosurgery	MICU	Cerebrospinal fluid	Brain tumor	Survived	06/12/96
96-5	37	M	Internal Medicine	MICU	Pus	Renal failure	Expired	06/17/96
96-7	33	M	General Surgery	SICU	Drain site	Pancreatitis	Expired	07/09/96
96-9	19	M	Neurosurgery	MICU	Blood	Cerebral hemorrhage	Survived	07/23/96
96-11	59	F	Neurosurgery	SICU	Cathetertip	Cerebral hemorrhage	Survived	07/31/96
96-12	60	M	Neurosurgery	SICU	Blood	Cerebral hemorrhage	Survived	08/08/96
96-13	55	M	General Surgery	SICU	Blood	Hepatocellular carcinoma	Expired	08/14/96
96-14	36	M	General Surgery	SICU	Blood	Ulcer perforation	Expired	09/17/96
96-15	63	M	General Surgery	SICU	Wound	Esophageal carcinoma	Survived	09/24/96
96-16	67	F	Internal Medicine	MICU	Blood	Myocardial infarction	Survived	10/14/96
96-17	51	M	Internal Medicine	MICU	Sputum	Pneumonia	Survived	10/14/96
96-18	57	F	Internal Medicine	MICU	Pus	Acute myeloid leukemia	Expired	12/10/96
96-19	75	F	Neurosurgery	SICU	Sputum	Cerebral hemorrhage	Survived	12/11/96
96-20	17	F	Neurosurgery	SICU	Transtracheal aspirate	Cerebral hemorrhage	Survived	12/14/96
2004								
04-1	89	M	Neurology	MICU	Blood	Parkinson disease	Survived	01/02/04
04-2	51	M	Thoracic Surgery	RICU	Blood	Angina pectoris	Survived	03/21/04
04-3	74	M	Internal Medicine	MICU	Blood	Myocardial infarction	Survived	04/12/04
04-4	73	F	Internal Medicine	MICU	Blood	Interstitial lung disease	Expired	04/26/04
04-5	32	M	General Surgery	SICU	Blood	Pancreas rupture	Survived	04/27/04
04-6	52	F	Internal Medicine	MICU	Blood	Gastric cancer	Expired	05/05/04
04-7	90	M	Internal Medicine	MICU	Blood	Myocardial infarction	Expired	05/10/04
04-10	58	M	Internal Medicine	MICU	Blood	Sepsis	Expired	05/24/04
04-11	86	M	Internal Medicine	MICU	Blood	Myocardial infarction	Survived	06/07/04
04-12	77	M	Thoracic Surgery	RICU	Blood	Angina pectoris	Survived	06/11/04
04-13	77	F	Internal Medicine	MICU	Blood	Pneumonia	Expired	06/19/04
04-14	66	M	General Surgery	SICU	Blood	Hepatocellular carcinoma	Survived	07/14/04
04-15	76	M	Internal Medicine	MICU	Blood	Pneumonia	Expired	07/20/04
04-16	43	F	Neurosurgery	SICU	Blood	Brain tumor	Survived	09/04/04
04-17	71	M	Internal Medicine	MICU	Blood	Myocardial infarction	Expired	09/05/04
04-18	77	M	Internal Medicine	MICU	Blood	Pneumonia	Expired	10/13/04
04-19	55	M	Internal Medicine	MICU	Blood	Gastric cancer	Expired	10/18/04

Abbreviations: SICU, surgical intensive care unit; MICU, medical intensive care unit; RICU, respiratory intensive care unit.

Table 2. Genotypic and phenotypic characteristics of MRSA isolates from ICUs in 1996 and 2004

No. of isolates	MLST		Antibiogram[a]										
	Allelic profile[b]	Sequence type (ST)	PEN	FOX	EM	GM	CIP	SXT	TC	TP	VM	CHL	RIF
1996													
96-2	1-32-1-1-4-4-3	254	R	R	R	R	R	S	R	S	S	S	R
96-3	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-4	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-5	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-7	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-9	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-11	1-1-1-1-1-1-1	1	R	R	R	R	R	S	R	S	S	S	S
96-12	1-32-1-1-4-4-3	254	R	R	R	R	R	R	R	S	S	S	R
96-13	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-14	1-32-1-1-4-4-3	254	R	R	R	R	R	R	R	S	S	S	R
96-15	1-1-1-1-1-1-1	1	R	R	R	R	R	S	S	S	S	S	S
96-16	1-4-1-4-12-1-10	5	R	R	S	R	R	S	R	S	S	S	S
96-17	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-18	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-19	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
96-20	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
2004													
04-1	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-2	1-4-1-8-4-4-3	72	R	R	S	S	S	S	S	S	S	S	S
04-3	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-4	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-5	1-4-1-4-12-1-10	5	R	R	R	R	R	S	S	S	S	S	S
04-6	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-7	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
04-10	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
04-11	1-4-1-4-12-1-10	5	R	R	R	R	R	S	R	S	S	S	S
04-12	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-13	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-14	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-15	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-16	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-17	2-3-1-1-4-4-3	239	R	R	R	R	R	R	R	S	S	S	S
04-18	1-4-1-4-12-1-10	5	R	R	R	R	R	S	S	S	S	S	S
04-19	1-4-1-4-12-1-10	5	R	R	R	R	R	S	S	S	S	S	S

[a]Antibiotic abbreviations: PEN, penicillin; FOX, cefoxitin; EM, erythromycin; GM, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; TC, tetracycline; TP, teicoplanin; VM, vancomycin ; CHL, chloramphenicol; RIF, rifampin; R, resistant; S, susceptible.
[b]Allelic profile in order of *arcC-aroE-glpF-gmk-pta-tpi-yqiL*.

isolates) and (2) 17 MRSA isolates in 2004 (all blood isolates). Organisms were identified by using conventional biochemical tests, the GPI Vitek card (bioMérieux, Hazelwood, MO, USA), and API20 Strep system (bioMérieux). The presence of the *mec*A gene was confirmed by PCR, as described previously[5], and the medical records of all 33 patients were reviewed retrospectively.

Antimicrobial susceptibility tests were performed using the disk diffusion method, according to the CLSI guidelines [6]. The antimicrobial agents tested included penicillin,

cefoxitin, erythromycin, gentamicin, ciprofloxacin, clindamycin, trimethoprim-sulfamethoxazole (SXT), tetracycline, teicoplanin, vancomycin, chloramphenicol, and rifampin.

Chromosomal DNA was extracted by using QIAamp DNA mini kits (QIAGEN GmbH, Hilden, Germany). MLST was performed according to the method described by Enright et al.[3]. The PCR fragments of seven genes (*arcC, aroE, glpF, gmk, pta, tpi, and yqiL*) were directly sequenced using an ABI Prism 3100 analyzer (Applied Biosystems, Foster City, CA, USA). Allele numbers at each of the seven loci defined the allelic profiles of each isolate, and these allelic profiles are described as sequence types (STs). The sequences obtained were compared with sequences at the MLST website (http://www.mlst.net/) to assign STs.

RESULTS

Table 1 provides the characteristics of the 33 ICU MRSA infections. The median ages were 52.5 years (17 - 67 years) in 1996 and 73 years (32 - 89 years) in 2004. Of the 33 patients, 6 of 16 patients in 1996 and 9 of 17 in 2004 died. Nine in 1996 and 13 in 2004 were male. Six and 10 MRSA strains were isolated from MICU and SICU, respectively, in 1996, 12 and 5 MRSA strains from MICU and SICU, respectively, in 2004.

According to the results of MLST analyses, the 33 MRSA isolates were classified into five ST types (Table 2). In 1996, the most frequent type was ST5 (n = 11, 68.7%), followed by ST254 (n = 3, 18.8%) and ST1 (n = 2, 12.5%), but in 2004, ST239 (n = 10, 58.8%) replaced ST5 as the most frequent type. The MRSA isolates tested were highly resistant to erythromycin (94%), gentamicin (97%), ciprofloxacin (97%), and tetracycline (85%), but the majority were susceptible to rifampin (91%). Only the ST254 clone was resistant to rifampin. The overall rate of resistance to SXT among the MRSA isolates tested was 36%. All 17 isolates belonging to ST5 were susceptible to SXT, whereas all ST-239 clones were resistant. No isolate was resistant to vancomycin, teicoplanin, or chloramphenicol.

DISCUSSION

MRSA is endemic within many hospitals worldwide. Several studies have found that ICUs have a higher incidence of MRSA than surgical or medical wards[7,8]. Moreover, ICUs have been proposed to have a central role in the intra- and inter-hospital spread of MRSA[8]. In this study, we compared MRSA strains isolated in 1996 and 2004 from ICUs using MLST and identified five ST types: ST1, ST5, ST72, ST239, and ST254, and clonal changes. Of the five STs identified, ST254 and ST1 were only found in 1996, and in particular, ST254 was identified only in patients hospitalized in surgical ICUs. In contrast, ST239 and ST72 were found only in 2004. ST5 was the most frequent type in 1996, but was less frequent in 2004, i.e., 68.7% in 1996 to 35.3% in 2004, and ST239, which was not detected in 1996, appeared as a major clone in 2004. ST5 and ST239 MRSAs were steadily isolated through the year of 1996 and 2004, respectively. This suggests that these ST types were endemic MRSA strains rather than outbreak strains. But, the possibility of occasional outbreaks in ICU could be considered because some patients with the same ST type MRSA occurred for a short period.

Previous epidemiologic studies of MRSA isolates in Asia using MLST have found ST239 the most prevalent, except in Korea and Japan[9], where most MRSA isolates was found to belong to ST5. Ko et al. suggested that the MRSA clones in Korea and Japan are completely different from those in other parts of the Asian region[9]. However, A few studies recently demonstrated increasing frequency of ST239 clone for a few years and suggested ST239 was a major clone in Korea[10,11]. Reasons for differences in prevalence of MRSA clone between each hospitals are unclear, though Cha et al. suggested the presence of different epidemic clones in hospitals located in different regions of Korea[11]. Data from this study revealed that the major lineage of MRSA from ICUs changed from ST5 in 1996 to ST239 in 2004. The major clones of MRSA were ST5 and ST239, which correspond with previous studies[10,11]. However, absence of ST239 clone in 1996 indicated the possibility that ST239 clone appeared later than ST5 clone.

In our study, all MRSA strains belonging to ST239 were resistant to SXT and tetracycline. These results correspond with the previous study from Korean hospital[11], however, differ from other Asian countries showing various ranges of susceptibilities[9]. Because our data result from a relatively small number of strains and from only a single hospital, it is necessary to build up more database to confirm the characteristics of ST239 clone from Korea.

In summary, our study demonstrates temporal clonal changes among MRSA isolates from ICUs between 1996 and 2004. Using MLST, we were easily able to compare the genetic diversities of endemic MRSA clones between these two study years.

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Multilocus Sequence Typing of Clonal Changes of Methicillin-resistant *Staphylococcus aureus* Isolated from Intensive Care Unit Patients: 1996 versus 2004

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for more than 70% of *S. aureus* isolates from tertiary-care hospitals in Korea. Recently, a multilocus sequence typing (MLST) scheme has been used to study the local and global epidemiologies of MRSA. The aim of this study is to compare the genetic background of MRSA strains isolated in the same ward during two different periods.

Methods: To investigate clonal changes of endemic MRSA isolates between 1996 and 2004, we studied a total of 33 MRSA strains (16 from 1996 and 17 from 2004) isolated in the intensive care units of a tertiary-care hospital in Korea. The isolates were analyzed for their sequence types by MLST and for their antimicrobial susceptibilities by the disk diffusion method.

Results: ST5 was the most frequent type (n=11, 68.7%) in 1996, followed by ST254 (n=3, 18.8%) and ST1 (n=2, 12.5%). In 2004, ST239 was the most frequent type (n=10, 58.8%), followed by ST5 (n=6, 35.3%).

Conclusion: The major clone type of MRSA isolates from intensive care unit patients changed from ST5 in 1996 to ST239 in 2004.

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Keywords: Methicillin-resistant *Staphylococcus aureus,* Multilocus sequence typing, ST5, ST239

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