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Original Article

Change in genotype of methicillin-resistant *Staphylococcus aureus* (MRSA) affects the antibiogram of hospital-acquired MRSA☆

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ABSTRACT

Recently, the dissemination of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) into hospitals has frequently been reported worldwide. Hospital-acquired MRSA (HA-MRSA) strains exhibit high-level resistance to multiple antimicrobial agents, whereas CA-MRSA strains are usually susceptible to non-β-lactams. Thus, it is predicted that the antibiogram of the HA-MRSA population would change along with the change in genotype of MRSA. Here, we investigated the changes in the MRSA population along with the MRSA antibiogram in a hospital between 2010 and 2016. Staphylococcal cassette chromosome (SCC) *mec* typing showed that the predominant HA-MRSA strains in the hospital dramatically changed from SCC*mec* type II, which is the major type of HA-MRSA, to SCC*mec* type IV, which is the major type of CA-MRSA. Multilocus sequence typing revealed that the predominant SCC*mec* type IV strain was a clonal complex (CC) 8 clone, which is mainly found among CA-MRSA. Furthermore, the CC1-SCC*mec* type IV (CC1-IV) clone significantly increased. Both the CC8-IV and CC1-IV clones exhibited high antimicrobial susceptibility. The antibiogram change of the HA-MRSA population was consistent with the antimicrobial susceptibilities and increased prevalence of the CC8-IV and CC1-IV clones. Our data reveal that the change in the genotypes of MRSA strains could impact the antibiogram of HA-MRSA population.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) exhibits resistance to multiple antimicrobial agents, and is one of the most common nosocomial pathogens [1]. MRSA strains are divided into two types: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA), which are mainly isolated from hospitalized patients and outpatients, respectively [2]. The methicillin resistance gene *mecA* is located on the staphylococcal cassette chromosome (SCC), and SCC*mec* can be classified into 11 types depending on the combination of *mecA* and *ccr* complexes [3,4]. In Japan, the main HA-MRSA strains comprise SCC*mec* types I, II, or III, whereas the main CA-MRSA strains are SCC*mec* type IV or V [5,6]. In general, HA-MRSA strains exhibit high-level resistance to multiple non-β-lactam antimicrobial agents such as quinolones, aminoglycosides, and macrolides [7]. By contrast, CA-MRSA strains are usually susceptible to non-β-lactams, whereas they produce various virulence and colonization factors, such as exfoliative toxin, Panton-Valentine leukocidin (PVL), and arginine catabolic mobile element (ACME) [8,9]. Thus, the pathogenicity of CA-MRSA strains is considered to be higher than that of HA-MRSA strains [10].

A NewYork/Japan clone of sequence type 5-SCC*mec* type II (ST5-II) has been found mainly among HA-MRSA strains isolated in the USA, Korea, and Japan [6,11,12]. The predominant CA-MRSA strain in the USA is the PVL-positive ST8-IV USA300 clone [13,14]. Currently, USA300 clones, which have both *arc* and *opp* clusters of ACME (ACME type I), are widely disseminated in both community and healthcare settings and have become worldwide pandemic clones [15]. In contrast, the ST59-IV, ST59-V, ST30-IV, and ST30-V clones are the predominant CA-MRSA strains in Asia [16,17]. Although the prevalence of PVL-positive CA-MRSA, including the

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USA300 clone, is very limited in Japan [18–20], the ST8 CA-MRSA clone has been increasing in the Japanese community [17,21].

Dissemination of CA-MRSA into hospitals has been frequently reported in recent years [22]. CA-MRSA is mainly associated with skin and soft tissue infections, but occasionally with invasive infections (such as bacteremia and necrotizing pneumonia), even in healthy individuals, including children and adolescents [22]. Thus, dissemination of the USA300 clone into hospitals will cause serious outbreaks, because there are many immune-compromised patients. In addition, it is expected that this increase in CA-MRSA prevalence will result in a change in the antibiogram of HA-MRSA population. Here, we investigated the transition of the MRSA population and associated antibiogram in hospital between 2010 and 2016. We focused on the particular clone types of CA-MRSA that have the largest contribution to the change in the HA-MRSA population. These results are expected to provide a scientific foundation for the status of HA-MRSA and the potential threat of CA-MRSA in hospital settings in Japan.

2. Materials and methods

2.1. Patients and bacterial strains

The study protocol was approved by The Ethics committee of The Jikei University School of Medicine for Biomedical Research (No. 28-013 (8256)). Informed consent was not required from the original patients as they had no clinical involvement in this study. All 792 patients with MRSA infections in our hospital between July 2010 and May 2016 were selected for this study. Among them, 40 patients (31 duplicated patients, seven outpatients, and two patients without corresponding clinical data) were excluded. Of the 752 MRSA strains, 18 strains were omitted from this study because of lack of *mecA* gene. Additionally, 134 MRSA strains were omitted because of non-HA-MRSA strains (sampling timing of ≤48 h after admission) [23]. Hence, 600 MRSA strains were used in this study. During this study period, the proportion of new MRSA isolation was decreased annually: 4.96% (2010), 3.97% (2011), 3.71% (2012), 3.1% (2013), 2.07% (2014), 2.27% (2015), and 1.83% (2016). The N315 MRSA strain and the JCSC6774 MRSA strain were used as reference strains of ST5-II New York/Japan clone and ST8-IV USA300 clone, respectively [24,25]. The following were used as *S. aureus* SCC*mec* type strains: NCTC10442 (type I), N315 (type II), 85/2082 (type III), JCSC4744 (type IV), and WIS (type V) [24]. *S. aureus* ATCC29213 was used as the quality control strain for antimicrobial susceptibility testing.

2.2. Analysis of clinical data

Name, ID, age, gender, clinical department, and MRSA isolation date of the patients were extracted from the clinical database stored at the Central Clinical Laboratory of our university. The birth date, admission date, discharge date, and mortality of the patients were extracted from the ordering system.

2.3. Bacterial growth condition and MRSA identification

All strains were cultured on tryptone soy agar (Oxoid, Hampshire, UK) under aerobic conditions at 35 °C for 24 h. *S. aureus* was identified by a positive Gram stain, proliferation on mannitol salt agar (Oxoid), production of coagulase (PS LATEX; Eiken Chemical, Tokyo, Japan), and detection of the *nuc* gene [26]. Strains that could not be identified by PCR were determined by sequencing of the 16S rRNA gene [27]. MRSA strains were identified by PCR-based detection of the *mecA* gene [28].

2.4. PCR amplification

Samples were prepared from a single MRSA colony suspended in 100 μL sterile water [28]. PCR assays for detecting the *mecA*, *tst* (encoding toxic shock syndrome toxin-1), *lukS/F-PV* (encoding PVL), and the ACME *arcA* and *opp-3C* genes were performed as described previously [6].

2.5. Molecular epidemiological analysis by SCCmec typing and multilocus sequence typing (MLST)

SCC*mec* typing and MLST were performed as described previously [24,29]. Isolates not identified as SCC*mec* types I to V were classified as nontypeable (NT).

2.6. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by an agar double-dilution method, according to the Clinical and Laboratory Standards Institute guidelines [30]. The following antimicrobial agents were used: ampicillin (Wako Pure Chemical Industries, Osaka, Japan), oxacillin (Sigma-Aldrich, Tokyo, Japan), cefotaxime (Wako), levofloxacin (Wako), clarithromycin (Tokyo Chemical Industry, Tokyo, Japan), clindamycin (Tokyo Chemical Industry), gentamicin (Wako), minocycline (Tokyo Chemical Industry), sulfamethoxazole-trimethoprim (Wako), vancomycin (Wako), teicoplanin (Sanofi, Paris, France), arbekacin (Meiji Seika Pharma, Tokyo, Japan), and linezolid (Pfizer, New York, USA). The breakpoints of these antimicrobial agents were defined using the Clinical and Laboratory Standards Institute interpretation criteria [31]. Undefined breakpoints were defined as described in a previous study [32].

2.7. Antimicrobial use density (AUD)

The AUD in The Jikei University Hospital between January 2010 and December 2016 was defined as the daily dose of antimicrobials used per 1000 patient-days as recommended by WHO [33]. The antimicrobial agents were categorized as follows: penicillin (penicillin G, ampicillin/cloxacillin, ampicillin, and sulbactam/ampicillin), piperacillin (piperacillin and tazobactam/piperacillin), first-generation cephalosporin (cefazolin), second-generation cephalosporin (cefotiam, cefmetazole, and flomoxef), third-generation cephalosporin (ceftazidime, ceftriaxone, cefotaxime, and sulbactam/cefoperazone), fourth-generation cephalosporin (cefozopran and cefepime), carbapenem (biapenem, panipenem/betamipron, imipenem/cilastatin, meropenem, and doripenem), monobactam (aztreonam), anti-MRSA drug (vancomycin, teicoplanin, arbekacin, linezolid, and daptomycin), fluoroquinolone (ciprofloxacin, pazufloxacin, and levofloxacin), aminoglycoside (kanamycin, streptomycin, amikacin, gentamicin, tobramycin, dibekacin, isepamicin, and spectinomycin), lincosamide (clindamycin and quinupristin/dalfopristin), tetracycline (minocycline), fosfomycin, and macrolide (erythromycin and azithromycin).

2.8. Statistical analysis

Differences in the rates of SCC*mec* types and antimicrobial resistance were evaluated using the χ^2 or Fisher's exact ($n < 10$) tests. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical features of patients

The clinical backgrounds of the patients included in this study are summarized in Table 1. The median age was 67, and the

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