Recurrence of heterogeneous methicillin-resistant *Staphylococcus aureus* (MRSA) among the MRSA clinical isolates in a Japanese university hospital

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Objectives: In the early 1980s, heterogeneous methicillin-resistant *Staphylococcus aureus* (hetero-MRSA) strains were predominant in the University of Tokyo Hospital. But, in the 1990s, they were completely substituted by homogeneously highly methicillin-resistant *S. aureus* (homo-MRSA) strains. Since 2000, however, we started observing an increase in MRSA strains with low cefazolin MICs (MRCLSA). This study was performed to understand the phenomenon by characterization of the 'cefa-zolin-susceptible' MRSA strains.

Methods: A total of 39 MRCLSA strains were collected between July 2000 and June 2002 and compared with 10 homo-MRSA strains isolated during the same period for their antibiograms and genotypes. The strains were also compared with the hetero-MRSA strains isolated in the same hospital in the early 1980s.

Results: In contrast to the homogeneous genotype [multilocus sequence type 5 and SCCmec type II.1 (Ila)] and multiresistant nature of the homo-MRSA strains, the MRCLSA strains were composed of various genotypes as revealed by multilocus sequence typing and SCCmec typing and had resistance only to a limited number of antibiotics. Most of the MRCLSA strains were also genetically differentiated from the hetero-MRSA strains of the 1980s. However, population analysis revealed that all of the MRCLSA strains were classified as hetero-MRSA strains.

Conclusions: A new group of hetero-MRSA strains genetically distinct from those dominant in the same hospital in the early 1980s might have emerged in the community and started invading the university hospital. This phenomenon may be caused by the change in the pattern of antibiotic use.

Keywords: community-associated MRSA, healthcare-associated MRSA, β-lactam antibiotics, population analysis

Introduction

In Japanese hospitals, heterogeneous methicillin-resistant *S. aureus* (hetero-MRSA) strains were frequently found in the early 1980s. However, after introduction of imipenem in 1987, almost all MRSA strains isolated in Japanese hospitals became homogeneously highly methicillin-resistant (homo-MRSA). In Japan, healthcare-associated MRSA (HA-MRSA) strains have exclusively been the strains expressing homogeneous methicillin resistance since 1990. However, in 2000, we started to notice the presence of a small percentage (3.2%) of MRSA strains with characteristically low cefazolin MIC values (1–16 mg/L) within the HA-MRSA strains in the University of Tokyo Hospital (UTH). In order to know the nature of the MRSA strains isolated in the 2000s in more detail, we collected a total of 39 MRSA strains with low cefazolin MICs (MRCLSA) and compared them with 10 typical homo-MRSA strains isolated from UTH.
Materials and methods

Bacterial strains

A total of 39 MRCLSA strains isolated from the laboratory of UTH between July 2000 and June 2002 were used in this study. Ten homo-MRSA strains used as control strains were isolated from separate inpatients of UTH in the corresponding period.

Antibiotic susceptibility test

MICs were determined according to the agar dilution method. Population analysis was performed as described previously. The number of resistant cells was calculated and plotted on a semi-logarithmic graph.

Molecular epidemiological characterization

SCCmec types were determined as described previously. To identify the subtype IV.5 (IVg), a primer set 4g1 (5'-ACTCATTGAAGTTCAACCC-3') and 4g2 (5'-AACACTGATTACATCTTCG-3') was additionally used. Multilocus sequence typing (MLST), PFGE and coagulase typing were performed as described previously. The genes related to pathogenicity were identified by PCR.

Determination of the nucleotide sequences: mecA gene complex of JCSC4744 and SCCmec elements of JCSC4788, JCSC4796 and JCSC4774

The DNA fragments covering the mecA gene complex of JCSC4744 and the SCCmec elements (from mecA gene to J1 region) of JCSC4788 and JCSC4796 were amplified by long-range PCR with several sets of primers as follows: mA10 (5'-GGGAGAAGTAAAACACTTTATT-3') and mA1, mA2 and is4, and mcR8 and cLm1. For the J1 region, the primer set a5 and cL2 was used with JCSC4744 and JCSC4796, and two primer sets [f1-16 (5'-TAGGTGCTGT TTAAGCACTCA-3') and 219-e4 (5'-ACATGTACCTCTTATTAG T-3') and cLnm1 (5'-AGCTAACAATTTGCGTTACTA-3') and psj21-3 (5'-TATCTTTAAGCGTTGACACAT-3')] were used with JCSC4796. The DNA fragment covering the mecA gene complex of JCSC4774 was also amplified by long-range PCR with mA10 and 3-632 (5'-GGTGAATATCGTTTACT-3'). These long-range PCR products were used for nucleotide sequencing.

Nucleotide sequence accession numbers

The nucleotide sequences of the SCCmec elements of JCSC4744, JCSC4788 and JCSC4796 have been deposited in the DDBJ/EMBL/GenBank databases under accession numbers AB266531, AB266532 and AB266533, respectively.

Results

Antibiotic susceptibility

Table 1 shows the MICs for tested representative strains. Most of the MRCLSA strains were resistant to all of the tested cephalosporins except for cefazolin. All MRCLSA strains had low imipenem MICs (≤1 mg/L), whereas most of them had MIC values beyond breakpoints for penicillins. Approximately 40% of them were found to be resistant to levofloxacin. The majority of them were resistant to gentamicin and macrolides. In contrast, homo-MRSA strains were resistant to all of the tested antibiotics other than the anti-MRSA antibiotics. To evaluate the apparent cefazolin susceptibility in more detail, we performed population analysis using five MRCLSA strains that were selected based on the difference in cefazolin MIC values. Figure 1 clearly shows that MRCLSA strains contained cefazolin-resistant (MIC >32 mg/L) subpopulations at a frequency of one in 10^5-10^6. The same was true with imipenem susceptibility (data not shown). Therefore, the apparent susceptible MIC values were interpreted as a manifestation of the heterogeneous nature of methicillin-resistant expression of these strains. On the other hand, all of the tested control HA-MRSA strains showed homogeneous cefazolin-resistant phenotypes.

Molecular epidemiological characterization

The genotype of the hetero-MRSA strains (MRCLSA strains) turned out to be extremely diverse as shown below (Table 1). SCCmec typing showed that the hetero-MRSA strains carried SCCmec elements rarely found in Japanese hospitals in the 1990s. Type IV SCCmec was the most prevalent (22 strains), followed by type II SCCmec (11 strains) and type I SCCmec (6 strains). Types IV and II SCCmec were further classified into subtypes. Thirteen and eight strains carried type IV.1 (IVa) and type II.2 (Iib) SCCmec, respectively, which were the popular subtypes carried by community-associated (CA-MRSA) isolates in Japan.

In order to find out whether the low cefazolin MIC value was caused by any mutation in mecA and its regulatory genes, we determined the nucleotide sequence of the mec gene complex of JCSC4774 carrying type II nt SCCmec and the SCCmec elements of three representative type IV SCCmec strains, JCSC4744, JCSC4788 and JCSC4796. The nucleotide sequence turned out to be almost identical to that of N315, MW2, 81/108 and M03-68, respectively, with the nucleotide identity >99%. These data implied that the low cefazolin MIC value does not relate to any mutations in the mec gene complex. MLST genotyping showed that the hetero-MRSA strains belonged to 11 different sequence types, which were classified into five clonal complexes. It was noted that most of them belonged to clonal complexes that had previously been identified in CA-MRSA strains, CC1, CC8, CC30 and CC91. On the other hand, all 10 homo-MRSA strains belonged to CC5, which is the dominant CC identified in HA-MRSA in Japan.

Coagulase typing showed that type I coagulase was the most prevalent (21 strains) among the hetero-MRSA strains, followed by type III (8 strains), type II (7 strains), type VII (2 strains) and type IV coagulase (1 strain). On the other hand, all 10 homo-MRSA strains harboured type II coagulase. agr specificity groups were well correlated with coagulase types. This distribution of coagulase type was completely different from that of the hetero-MRSA strains isolated in the early 1980s at UTH, in which type IV coagulase was the most prevalent. These data indicated that the hetero-MRSA strains isolated in the early 2000s were clonally different from the hetero-MRSA strains isolated in the early 1980s.

The pattern of the genes related to pathogenicity showed that neither the Panton–Valentine leucocidin (PVL) gene nor the exfoliative toxin A gene (eta) was identified among the hetero-MRSA strains of the 2000s, although 45.3% of the
Table 1. Susceptibilities and molecular epidemiological characteristics of representative strains of 39 hetero-MRSA strains isolated in the 2000s and reference strains

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Dark grey shading, resistant; light grey shading, intermediate; no shading, susceptible.

Category: E2, hetero-MRSA isolated in the 2000s; E-, hetero-MRSA; O, homo-MRSA.

CFS, ceftazidime; ZOX, cefoxitin; CDR, ceftinil; CDN, cephradine; CRO, ceftriaxone; AMP, ampicillin; SAM, ampicillin/sulbactam; AMX, amoxicillin; OXA, oxacillin; IPM, imipenem; GEN, gentamicin; ERY, erythromycin; CLR, clarithromycin; AZM, azithromycin; MIN, minocycline; TET, tetracycline; LVX, levofloxacin; LZD, linezolid; TEC, teicoplanin; VAN, vancomycin.

emu, collagen-binding protein gene.

e, enterotoxin A gene.

f, enterotoxin B gene.

Ist, enterotoxin toxin A gene.

LukS-LukF, PVL gene.

nt, non-typeable.
hetero-MRSA strains isolated in the early 1980s in Japan were PVL-positive. Two strains carried the \textit{tst}-1 gene, which is commonly carried by Japanese HA-MRSA, whereas all 10 homo-MRSA strains harboured the \textit{tst}-1 gene.

Discussion

\textit{S. aureus} clinical strains isolated in 1982 and 1992 at UTH were studied by Tanaka \textit{et al}. They described hetero-MRSA strains with low cefazolin MICs among the hetero-MRSA strains isolated in 1982. One of the strains from the 1980s with a low cefazolin MIC (strain 20-1) belonged to pre-MRSA. Pre-MRSA is an \textit{S. aureus} carrying a \textit{mecA} gene but its expression is strongly repressed by the accompanying intact copy of a \textit{mecI} gene. Among the MRCLSA strains, JCSC4773 and JCSC4747 carried type II coagulase and SCC\textit{mec} type II.1, in common with pre-MRSA strains. Nucleotide sequencing revealed that \textit{mecI} genes of JCSC4773 and JCSC4747 were identical with the intact \textit{mecI} of N315, the prototype of pre-MRSA. Furthermore, prior exposure of JCSC4773 to cefzoxime caused expression of cefazolin resistance (data not shown). These data indicated that the two strains among the 39 MRCLSA strains were pre-MRSA strains. Since exposure of pre-MRSA to \(\beta\)-lactam antibiotics frequently selects out the \textit{mecI}-mutated hetero-MRSA sub-strains, it is probable that the two pre-MRSA strains had remained unexposed to \(\beta\)-lactam antibiotics in the University Hospital, i.e. they were generated in the community by acquiring an SCC\textit{mec} with the intact \textit{mecI} gene, and were recently carried into the hospital by the admitted patients. The community origin of the hetero-MRSA strains of the 2000s is also implicated by the fact that 82% of the hetero-MRSA strains belonged to clonal complexes CC1, 8, 30 and 91 that had previously been identified in CA-MRSA strains.

Since 1991, when parenteral use of vancomycin was officially approved, \(\beta\)-lactam antibiotics have not been widely used to treat MRSA infections in Japan. This decrease in \(\beta\)-lactam antibiotic use might have allowed CA-MRSA to come into and survive in the hospitals and caused recurrence of hetero-MRSA strains in the hospitals. Most of the hetero-MRSA strains were genetically distinct from those dominant in the early 1980s and from typical HA-MRSA strains, i.e. they are a new group of hetero-MRSA strains. In Japan, we are increasingly aware of the emergence of CA-MRSA strains in the community. If the trend continues, we would expect to see in the near future a further increase in hetero-MRSA strains of community origin invading Japanese hospitals as reported in France and Australia a decade ago.

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Transparency declarations

None to declare.

References


