Superior in vitro activity of carbapenems over anti-methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and some related antimicrobial agents for community-acquired MRSA but not for hospital-acquired MRSA

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\textbf{Abstract} Eighty-eight strains of Panton–Valentine leukocidin (PVL)-positive and -negative community-acquired methicillin-resistant \textit{Staphylococcus aureus} (CA-MRSA) and 152 strains of hospital-acquired MRSA (HA-MRSA) were examined for susceptibility to carbapenems, oxacillin, and other antimicrobial agents. CA-MRSA strains were more susceptible to carbapenems (MIC\(_{50}\), 1–4 \(\mu\)g/ml) than HA-MRSA strains (MIC\(_{50}\), 32–64 \(\mu\)g/ml). Among the carbapenems examined, CA-MRSA strains were most susceptible to imipenem (MIC\(_{50}\), 0.12 \(\mu\)g/ml; MIC\(_{90}\), 1 \(\mu\)g/ml). A similar tendency was observed with oxacillin, but less markedly (MIC\(_{50}\), 32 \(\mu\)g/ml for CA-MRSA and \(\geq\)256 \(\mu\)g/ml for HA-MRSA). This difference was also observed between CA-MRSA and HA-MRSA in susceptibility levels to cephems, erythromycin, clindamycin, and levofloxacin, but not to ampicillin, vancomycin, teicoplanin, linezolid, and arbekacin. The data indicate that, in terms of MIC\(_{50}\) or MIC\(_{90}\) values, CA-MRSA is 64–256 times more susceptible to imipenem than HA-MRSA, and for CA-MRSA, some carbapenems, e.g., imipenem, show better in vitro activity than anti-MRSA or some related agents.

\textbf{Key words} Community-acquired methicillin-resistant \textit{Staphylococcus aureus} (CA-MRSA) · Antimicrobial activity · Carbapenems · Oxacillin

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is generated from \textit{S. aureus} by the acquisition of staphylococcal cassette chromosome mec (SCCmec).\(^1\) Since its isolation in 1961, MRSA has been a major cause of nosocomial infections.\(^2\) Such MRSA is also called hospital-acquired MRSA (HA-MRSA) and is multidrug-resistant in most cases, and several pandemic clones, including the New York/Japan clone (with multilocus sequence type [ST] 5 and SCC\textit{mec}I[ST5:SCC\textit{mec}II]) and the Hungarian clone (with ST239: SCC\textit{mec}III), have been identified and characterized.\(^3\)

More recently (since the late 1990s), another type of MRSA, community-acquired MRSA (CA-MRSA), which often carries genes for Panton–Valentine leukocidin (PVL), has been noted as an emerging pathogen.\(^4,5\) In contrast to HA-MRSA, CA-MRSA is associated with skin and soft-tissue infections (SSTIs) in children and athletes in the community.\(^6\) It is also associated with life-threatening infections such as pneumonia, sepsis, pelvic abscesses, and necrotizing fasciitis.\(^7\) PVL-positive CA-MRSA involves several clones, such as CA-MRSA ST1:SCC\textit{mec}IV (known as USA400) and ST8:SCC\textit{mec}IV (known as USA300 clone), mainly found in the United States,\(^7\) CA-MRSA ST80:SCC\textit{mec}IV, mainly found in Europe,\(^7\) and CA-MRSA ST30:SCC\textit{mec}IV, which is found worldwide.\(^8\) PVL-positive CA-MRSA from various countries or areas is susceptible to all non-\(\beta\)-lactam antimicrobial agents,\(^9,10\) but other PVL-positive CA-MRSA clones, such as USA300, are becoming multidrug-resistant.\(^7\)

In this study, we examined the in vitro susceptibilities of PVL-positive and -negative CA-MRSA to carbapenems, oxacillin, and other antimicrobial agents, in comparison with HA-MRSA.

In this study, MRSA was defined as \textit{S. aureus} showing MICs (\(\geq\)4 \(\mu\)g/ml) of oxacillin, or \textit{S. aureus} positive for the \textit{mec}A gene, according to the Clinical and Laboratory Standards Institute (CLSI).\(^5\) CA-MRSA was defined as MRSA isolated from outpatients who had no history of hospitalization within the past year, and presented with no other established risk factors for HA-MRSA infections, such as surgery, residence in a long-term care facility, dialysis, or in-dwelling percutaneous medical devices and catheters.\(^8\) HA-MRSA was defined as MRSA from patients isolated 48 h or later after hospitalization (and presenting with established risk factors for HA-MRSA infections as described above).

A total of 240 strains (88 strains of CA-MRSA and 152 strains of HA-MRSA) were examined. CA-MRSA strains were all \textit{mec}A-positive, and included PVL-positive strains (50 strains) with ST1:SCC\textit{mec}IV (\(n = 1\)), ST8:SCC\textit{mec}IV
whose ST type has not been characterized (n = 2), ST30:SCCmecIV (n = 12), ST59:SCCmecVII (n = 30), and ST80:SCCmecIV (n = 3), as well as those whose ST type has not been characterized (n = 2) from Japan, Taiwan, Russia, the United States, Australia, the Netherlands, and France, and including those reported previously.8,10,11 PVL-negative CA-MRSA strains (38 strains) included those with ST8:SCCmecIV (n = 8), ST88:SCCmecIV (n = 1), ST89:SCCmecIV (or other SCCmec types) (n = 15), and ST91:SCCmecIV (n = 3), as well as those whose ST type has not been characterized (n = 11) and which were isolated from 2003 to 2007 from patients with SSTIs in Niigata, Tokyo, Chiba, Saitama, Yokohama, Tochigi, and Aichi, Japan. These included MRSA from patients with SSTIs reported previously.11

HA-MRSA strains (SCCmecII in 91.1%) included 28 strains from infants in a neonatal intensive care unit (NICU), and 107 strains isolated from blood, sputum, urine, etc. of other hospitalized patients in Niigata, Tokyo, Kanagawa, Chiba, and Saitama, Japan, from 2003 to 2007. They contained MRSA with ST5:SCCmecII (a major New York/Japan clone in Japan) and ST764 (a single-locus variant of ST5):SCCmecII (a minor clone in Japan), reported previously.11,12 HA-MRSA strains also included those with ST239:SCCmecIII (Hungarian clone, n = 10), ST5:SCCmecII (New York/Japan clone, n = 2), and ST59:SCCmecIV (or other SCCmec types) (PVL-negative, n = 5) from Taiwan, including those reported previously.11 All HA-MRSA strains were negative for PVL.

Susceptibility testing of bacterial strains was performed by the agar dilution method with Mueller–Hinton agar (Difco, Sparks, MD, USA) according to previous procedures.9 The final concentrations of antimicrobial agents were from 0.001 to 128 μg/ml. Antimicrobial agents were donated by their manufacturers. S. aureus ATCC29213 was used as a reference strain for quality control.9 Breakpoints for drug resistance were those described by the CLSI.9

The MIC data of antimicrobial agents against CA-MRSA and HA-MRSA strains are summarized in Table 1. As for oxacillin resistance, CA-MRSA strains manifested lower levels of resistance (MIC50, 16 μg/ml; MIC90, 32 μg/ml), in contrast to those for HA-MRSA strains (MIC50, 128 μg/ml; MIC90, ≥256 μg/ml). This tendency was more sharply demonstrated with carbapenem susceptibilities. The susceptibility levels (MIC50 and MIC90) of carbapenems for CA-MRSA strains were 0.12–1 μg/ml and 1–4 μg/ml, respectively. In contrast, those for HA-MRSA strains were 32 μg/ml and 32–64 μg/ml, respectively. Among carbapenems, CA-MRSA strains manifested the highest susceptibility to imipenem (MIC50, 0.12 μg/ml; MIC90, 1 μg/ml). Regarding PVL-positive and -negative CA-MRSA strains, PVL-positive strains tended to be more susceptible to oxacillin and imipenem than PVL-negative strains (Table 1).

In sharp contrast, the susceptibility (or resistance) levels of CA-MRSA strains for ampicillin (MIC50, 16 μg/ml; MIC90, 32 μg/ml) and anti-MRSA or related agents (arbekacin (MIC50, 0.5 μg/ml; MIC90, 2 μg/ml), vancomycin (MIC50, 1 μg/ml; MIC90, 1 μg/ml), teicoplanin (MIC50, 1 μg/ml; MIC90, 1 μg/ml), and linezolid (MIC50, 1 μg/ml; MIC90, 2 μg/ml)) were the same as, or very similar to, those of HA-MRSA strains (Table 1). Therefore, for CA-MRSA strains (but not HA-MRSA strains), some carbapenems (e.g., imipenem) showed better in vitro activity than anti-MRSA or related agents examined (Table 1).

When MIC values of imipenem for CA-MRSA strains were plotted against those of oxacillin or ampicillin for CA-MRSA strains, a relatively good correlation coefficient (R2 = 0.7794) was observed between imipenem and oxacillin, while no correlation was observed between imipenem and ampicillin (R2 = 0.3654).

The in vitro susceptibility of CA-MRSA and HA-MRSA strains to cephems, erythromycin, clindamycin, and levofloxacin were also examined (Table 1). CA-MRSA strains were more susceptible to those antimicrobial agents than HA-MRSA strains. The susceptibility level of CA-MRSA strains for imipenem was highest among β-lactam antimicrobial agents, but was lower than that for levofloxacin.

The CLSI instructed that for MRSA, MIC results for β-lactam agents, including carbapenems, should be reported as resistant or should not be reported, because most cases of documented MRSA infections have responded poorly to β-lactam therapy, or because convincing clinical data have yet to be presented that document the clinical efficacy of those agents.9 In particular, for CA-MRSA (an emerging pathogen), clinical data on carbapenems, including imipenem, are lacking.

This study has demonstrated that CA-MRSA strains, but not HA-MRSA strains, are highly susceptible to carbapenems, especially imipenem, in vitro. The difference in susceptibility levels for imipenem between CA-MRSA and HA-MRSA strains was 64–256 times in terms of MIC50 or MIC90 values. As for CA-MRSA, the MIC50 and MIC90 values of imipenem were similar to, or even greater than, those of anti-MRSA or some related agents (arbekacin, vancomycin, teicoplanin, and linezolid).

Regarding oxacillin resistance, it has been reported that CA-MRSA strains manifest lower levels of resistance than HA-MRSA strains in many cases.10,11,13 This tendency was more sharply shown with imipenem susceptibility. Susceptibility (or resistance) levels of CA-MRSA strains for imipenem and oxacillin were correlated (indicating that both phenotypes are coded for by the meca gene), while no such correlation was observed between imipenem and ampicillin (whose resistance is mainly coded for by the β-lactamase gene, bла, on a penicillinase plasmid).4,14 Therefore, this study raises the critical question of how meca-mediated (imipenem and oxacillin) resistance is regulated. Although some meca gene variants have nonsynonymous substitutions, such amino acid substitutions do not seem to correlate with resistance levels.14

MRSA is generated from S. aureus by acquisition of the staphylococcal cassette chromosome mec (SCCmec).1 For HA-MRSA, it is considered that this genetic event occurred only a limited number of times, and the resultant MRSA strains have spread within hospitals world-wide as a pandemic clone. In contrast, it is considered that CA-MRSA has emerged more recently and has started to spread mainly in the community.4,5 It is possible that HA-MRSA strains have been selected in more cases and for a longer period.