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Original article

Microbiological and molecular epidemiological analyses of community-associated methicillin-resistant *Staphylococcus aureus* at a tertiary care hospital in Japan

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ABSTRACT

Molecular characterization of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is generally conducted referred to staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V. CA-MRSA is now a cause of concern since such strains have been isolated not only from individuals in a community but also from patients in healthcare settings. The aim of this study was to analyze microbiological and molecular epidemiological features of CA-MRSA strains at a Japanese tertiary care hospital using PCR based-open reading frame typing (POT). This technique allows for molecular classification into CA-MRSA (POT-CA) and hospital-associated (HA-) MRSA (POT-HA) with clonal discrimination. Clinical MRSA isolates obtained from consecutive patients between October 1, 2012 and September 30, 2013 at the hospital were analyzed in combination with the clinical definition for CA-MRSA by the Centers for Disease Control and Prevention and POT. Of 219 isolates (76 clonal groups), 64 (29.3%) were clinical-HA/POT-CA isolates (22 clonal groups). Some clones of them accumulated in this hospital and might be involved in nosocomial transmission. Virulent factors of the isolates were analyzed, and only one (1.6%) Panton-Valentine leukocidin gene positive isolate but no arginine catabolic mobile element genes positive isolate were found in clinical-HA/POT-CA. Additionally, clinical-HA/POT-CA isolates showed higher antimicrobial susceptibility than clinical-HA/POT-HA, especially to minocycline, doxycycline, and amikacin. The most frequent genotype of molecular CA-MRSA was multi-locus sequence type 5-SCC*mec*IV, previously not detected in Japan. Although CA-MRSA at this hospital showed low virulence and higher antimicrobial susceptibility, the risk of nosocomial infection from them should be recognized, requiring stricter infection control measures.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the major pathogen of multidrug-resistant organisms causing healthcare-associated infections (HAI) [1]. Recently, community-associated (CA-) MRSA has caused severe infections such as necrotizing pneumonia even in healthy individuals in the community, and as such has become a global threat [2]. CA-MRSA is defined by the Centers for Disease Control and Prevention (CDC), in brief, as MRSA from individuals without a certain history of healthcare exposures [3]. CA-MRSA is also further characterized by molecular aspects

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that are not seen in conventional hospital-associated (HA-) MRSA in that it harbors the staphylococcal cassette chromosome *mec* (SCC*mec*) IV or V, which results in better susceptibility to many antimicrobial agents [2]. It also contains the Panton-Valentine leukocidin (PVL) gene and the arginine catabolic mobile element (ACME) genes; the former is related to the virulence and the latter to the ability to colonize and spread [4,5]. CA-MRSA is now analyzed around the world in the context of these molecular features [6,7].

It was recently observed that CA-MRSA could be isolated from hospitalized patients, which is not necessarily consistent with the CDC definition, and is now a source of concern since it appears to be an HAI pathogen [8–10]. There have been reports of outbreaks in neonatal care units (NICU) [11,12] and hospital-onset bloodstream infection [13] caused by SCC*mec*IV-MRSA. However, a specific measure of infection control against CA-MRSA has been under development [10,14], therefore further microbiological, epidemiological, and clinical data about CA-MRSA in healthcare settings are required.

PCR based-open reading frame (ORF) typing (POT) is a molecular typing technique performed using multiplex PCR without nucleotide sequences. It is used to differentiate MRSA clonality by detecting specific ORFs lysogenized into bacterial chromosomal DNA [15,16]. POT can also be used to classify CA-MRSA and HA-MRSA by predicting SCC*mec* type [17]. This technique has high discriminatory power, similar to that of pulsed-field gel electrophoresis (PFGE) [15,17–19] or repetitive-sequence-based PCR [20]. In addition, POT features an easier procedure, lower cost, and shorter turnaround time, and has been applied to an epidemiological survey [17] and infection control in a hospital or a ward [21,22]. We conducted microbiological and molecular epidemiological analyses of MRSA isolated at a tertiary hospital in Japan. The aims of this study were: 1) to classify CA-MRSA and HA-MRSA according to clinical definition and molecular characteristics, 2) to investigate microbiological features related to virulence and antimicrobial susceptibility, and 3) to evaluate the genetic clonality and profiles of MRSA at Tohoku University hospital.

2. Materials and methods

2.1. Bacterial isolates, patient information, and ethics approval

Tohoku University Hospital is a teaching and tertiary care hospital in Japan with over 50 clinical departments and over 1200 beds. Between October 1, 2012 and September 30, 2013, we identified a total of 1500 MRSA isolates from patients in a total of 26 wards and 8 different outpatient-clinics at our hospital using the Vitek-2 System (Sysmex-bioMérieux, Tokyo) or the Microscan Walkaway System (Siemens Healthcare Diagnostics, Tokyo). We further selected 232 isolates as initial and non-duplicate isolates from consecutive patients. These 232 isolates were obtained from clinical specimens derived from various patient sites: 66 (28.4%) from sputum, 49 (21.1%) from wounds, 37 (15.9%) from nares, 19 (8.2%) from the lower respiratory tract, 13 (5.6%) from stool, 12 (5.2%) from the skin, 9 (3.9%) from throat, 7 (3.0%) from blood, 6 (2.6%) from urine, 5 (2.2%) from a central venous catheter, 4 (1.7%) from vaginal discharges, 3 (1.3%) from ear discharges, 1 (0.4%) from ascites, and 1 (0.4%) from eye discharges.

The bacterial DNA of the isolates was extracted using the Cica Geneus® DNA Extraction Reagent (Kanto Chemical Co. Inc., Japan). MRSA identification was confirmed by PCR using primers for each of the 16S ribosomal RNA (rRNA) [23], *nuc* [23], and *mecA* genes [24] as previously described, with slight modification. We identified 231 isolates as MRSA with positive results for each 16S-rRNA, *nuc*, and *mecA* gene. One isolate was identified as methicillin-susceptible *S. aureus* and was excluded from further analysis.

We collected the electronic medical records of patients from which isolates were obtained and identified relevant information including pre-hospital medical history, admittance date, the day the initial MRSA was obtained. The ethics committee of Tohoku University Graduate School of Medicine approved this retrospective study.

2.2. Clinical and POT classification for CA-MRSA and HA-MRSA

Based on the CDC's definition for CA-MRSA [3] and the collected patient information, we classified and designated isolates as “clinical CA-MRSA (cCA)” or “clinical HA-MRSA (cHA)”. cCA was defined as MRSA obtained from patients without any history of: 1) a positive MRSA culture from any body site obtained more than 48 h after admission to a hospital (if hospitalized), 2) a prior MRSA infection or colonization, 3) hospitalization, surgery or residency in a long-term care facility, hemodialysis, or peritoneal dialysis within the past year, and 4) current indwelling percutaneous devices or catheters; MRSA isolates that were isolated more than 48 h after admission were classified to cHA.

POT analysis was performed for all of the MRSA isolates using the Cica Geneus® Staph POT KIT (Kanto Chemical Co.) according to the manufacturer's instructions and previous studies [17,20,22]. The POT index of each isolate was subsequently determined for clonal discrimination. For the POT 1 index, one part of the POT index was determined from a combination of certain genetic components of SCC*mec* elements and could predict the type of SCC*mec*. Subjects were classified into CA-MRSA or HA-MRSA according to variations in these predictions [17] with slight modification (Supplemental Table), and thus further designated as ‘POT-CA (pCA)’, ‘POT-HA (pHA)’, or ‘POT-unpredicted (UP)’. In addition, specific SCC*mec* typing was also performed on subjects, as previously described [25,26], to confirm the results of POT prediction. The isolates whose SCC*mec* was not assigned to established types were designated as SCC*mec*-not assigned (NA). We also divided subjects into clonal groups by POT index and further designated the clonal groups with plural isolates as a ‘POT cluster’ and those with a single isolate as a ‘POT singleton’ for evaluating the clonal accumulation in this hospital.

Through combination of clinical classification and POT classification, excluding POT-UP and SCC*mec*-NA isolates, subjects were further classified into four groups: cCA/pCA, cCA/pHA, cHA/pCA, and cHA/pHA.

2.3. Detection of virulent factors

For investigation of virulent factors known to be associated with CA-MRSA, all of the MRSA isolates were tested for the presence of the PVL (*lukS/F-PV*) [27] and the ACME-related (*arcA* and *opp3AB*) genes [5] as previously described.

2.4. Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) was determined by micro-broth dilution according to the guidelines of the Clinical Laboratory Standard Institute (CLSI M100 S22) [28]. The following antimicrobial agents were used in the test: penicillin, oxacillin, ampicillin, sulbactam/ampicillin, cefazolin, cefoxitin, ceftriaxone, cefepime, imipenem, gentamicin, amikacin, minocycline, doxycycline, rifampicin, levofloxacin, clindamycin, erythromycin, sulfamethoxazole/trimethoprim, arbekacin, vancomycin, teicoplanin, and linezolid. The MIC_{50}, MIC_{90}, and percentage of non-susceptible (NS) isolates (intermediate-resistant and resistant, %NS) in each