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Epidemiological and molecular characteristics of meticillin-resistant *Staphylococcus aureus* in Turkey: A multicentre study

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ABSTRACT

The aim of this study was to investigate the epidemiological and molecular features of clinical meticillin-resistant *Staphylococcus aureus* (MRSA) isolates in Turkey. MRSA isolates were collected from six regions of Turkey. The *mecA* and *nuc* genes were detected by PCR. Antimicrobial susceptibilities were determined by the disk diffusion method. Staphylococcal cassette chromosome *mec* (SCC*mec*) and staphylococcal protein A (*spa*) typing were performed by the sequencing method for 270 randomly selected MRSA isolates. The US Centers for Disease Control and Prevention (CDC) definition was used for epidemiological diagnosis of community-associated MRSA (CA-MRSA). Resistance rates of MRSA to ciprofloxacin, gentamicin, clindamycin, erythromycin, rifampicin, trimethoprim/sulfamethoxazole and tetracycline were 93.4%, 81.2%, 38.5%, 57.8%, 93.9%, 1.1% and 93.1%, respectively. The most frequent SCC*mec* type was SCC*mec* III (91.1%). SCC*mec* type IV was found in 5.2% of the isolates. The most frequent *spa* type was t030 (81.1%). Five isolates were CA-MRSA if only the epidemiological definition was used (5/725; 0.7%). Two isolates were defined as CA-MRSA both by epidemiological features and SCC*mec* typing (2/270; 0.7%). Of 14 SCC*mec* type IV isolates, 12 were not defined as CA-MRSA by epidemiological features. In conclusion, this is the most comprehensive multicentre study in Turkey investigating MRSA using both epidemiological and genotypic features. The CA-MRSA rate is low in Turkey. Combined use of epidemiological and genotypic methods is the most accurate approach for the diagnosis of CA-MRSA.

1. Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen both in hospital and community settings. In addition to well-known healthcare-associated MRSA (HA-MRSA) infections, since the early 1990s community-associated MRSA (CA-MRSA) infections have been reported from different parts of the world. CA-MRSA causes infections in previously healthy young patients without prior healthcare contact and has different molecular features [1]. CA-MRSA isolates are usually more susceptible to non-β-lactam agents than HA-MRSA isolates [2].

According to the epidemiological definition of CA-MRSA, MRSA must be identified in the outpatient setting or within 48 h after admission to the hospital in a patient with no medical history of MRSA infection or colonisation, no medical history in the past year of hospitalisation, admission to a nursing home, dialysis or surgery, and no permanent indwelling catheters or medical devices [1,3]. However, because of difficulties in the epidemiological definition of CA-MRSA, it is proposed to combine a molecular typing method with the epidemiological definition [1].

There are several typing methods for MRSA, including staphylococcal cassette chromosome *mec* (SCC*mec*) typing, staphylococcal

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protein A (*spa*) typing, macrorestriction pattern analysis by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and multilocus variable-number tandem repeat analysis (MLVA) [4].

The *mecA* gene responsible for meticillin resistance is located on a mobile genetic element designated SCC*mec*. This element also contains cassette chromosome recombinase (*ccr*) genes [5]. Eleven SCC*mec* types have been described to date [6]. CA-MRSA usually carries SCC*mec* types IV or V, whereas HA-MRSA usually carries SCC*mec* types I–III [5].

Sequencing of the variable repeat X region of the *spa* gene is also a useful tool in typing of MRSA because of its rapidity, ease of use and standardised nomenclature [4,7].

It is important to know the characteristics of MRSA isolates both for epidemiological and clinical evaluations. The aim of this study was to investigate the epidemiological and molecular features of clinical MRSA strains from different parts of Turkey and to evaluate the different methods in the definition of CA-MRSA.

2. Materials and methods

2.1. MRSA isolates

Clinical MRSA isolates were collected from six university hospitals (Kocaeli, Ankara, Kayseri Erciyes, Adana Çukurova, Diyarbakır Dicle and Trabzon Karadeniz Technical Universities) in different regions of Turkey between the years 2005 and 2008. A comprehensive form including epidemiological, demographic and clinical information for the patients was also completed. The form included: age; sex; underlying diseases [diabetes, asthma, chronic obstructive pulmonary disease (COPD), hypertension, renal failure, dialysis, human immunodeficiency virus (HIV) positivity, coronary and skin disease, etc.]; addictions (smoking, alcohol, drugs); hospitalisation and operations in the last 2 years; presence of hospital staff in the family; presence of any probe, catheter or foreign bodies; use of antibiotics in the last year; which clinic the patient was admitted to; diagnosis; sample type; and the date on which the culture was taken.

Isolates sent from different centres were confirmed by DNase and oxacillin agar screen tests and were then stored at −80 °C. Isolates were passaged twice before the study.

2.2. Determination of mecA and nuc genes

Isolation of DNA from all isolates was performed on a BioRobot Workstation (QIAGEN, Hilden, Germany) using magnetic particle technology (Fluorion Mag 16; Iontek Molecular Diagnostics, Istanbul, Turkey), and the *mecA* and *nuc* genes were detected using a commercial PCR assay on a real-time platform (Fluorion MRSA QLS 1.0; Iontek Molecular Diagnostics).

2.3. Antimicrobial susceptibility testing

The susceptibilities of the MRSA isolates to ciprofloxacin, gentamicin, erythromycin, clindamycin, fusidic acid, linezolid, quinupristin/dalfopristin, rifampicin, trimethoprim/sulfamethoxazole (SXT), tetracycline, vancomycin, teicoplanin and mupirocin as well as inducible clindamycin resistance were detected by the Kirby–Bauer disk diffusion method and were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8].

2.4. Determination of SCCmec elements

SCC*mec* analysis was performed by real-time PCR for 270 randomly selected isolates representative of each centre [5]. The database at http://www.staphylococcus.net/was used for SCC*mec* typing.

2.5. spa typing

spa typing was also performed on the same 270 isolates as previously described [9]. The primers used were as follows: *spa*F1, GAC GAT CCT TCG GTG AGC-3 (nucleotides 1096–1113); and *spa*R1, CAG CAG TAG TGC CGT TTG C (nucleotides 1534–1516) [10]. Single-locus DNA sequencing of the variable repeat X region of the *spa* gene was used for discriminatory typing of MRSA by Ridom SpaServer (http://spaserver.ridom.de). *spa* typing was performed twice for untyped isolates.

2.6. Epidemiological definition of CA-MRSA

The US Centers for Disease Control and Prevention (CDC) definition was used for epidemiological diagnosis of CA-MRSA: diagnosis of MRSA made in the outpatient setting or by a culture positive for MRSA within 48 h after admission to the hospital; no medical history of MRSA infection or colonisation; no medical history in the past year of hospitalisation, admission to a nursing home, dialysis and surgery; and no permanent indwelling catheters or medical devices that pass through the skin into the body [3].

2.7. Statistical analyses

Statistical analyses were performed using IBM SPSS for Windows v.20.0 (IBM Corp., Armonk, NY). Kolmogorov–Smirnov tests were used to test the normality of data distribution. Continuous variables were expressed as the mean ± standard deviation, and categorical variables were expressed as percentages. Comparison of continuous variables between the two groups was performed by Student's *t*-test. Comparison of categorical variables between the two groups was performed using the Fisher's exact test and Monte Carlo χ^2 test. A two-sided *P*-value of <0.05 was considered statistically significant.

3. Results

A total of 725 non-duplicate MRSA were isolated from samples of blood and central venous catheter (25.0%), skin and soft tissue (16.6%), respiratory tract (15.2%), urinary tract (3.7%), sterile body fluids (2.8%) and other body sites (36.8%). The general characteristics of the patients enrolled in the study are listed in Table 1.

Resistance rates of MRSA to non-β-lactam antimicrobials were 93.4%, 81.2%, 38.5%, 57.8%, 93.9%, 1.1% and 93.1% to ciprofloxacin, gentamicin, clindamycin, erythromycin, rifampicin, SXT and tetracycline, respectively (Table 2).

Resistance rates to ciprofloxacin, gentamicin, erythromycin, rifampicin and tetracycline were significantly lower in SCC*mec* type IV isolates than in SCC*mec* type I–III isolates ($P < 0.05$) (Table 3).

The most frequent SCC*mec* type among the MRSA isolates was SCC*mec* III (91.1%). SCC*mec* type IV, which is known as the most prevalent SCC*mec* type in CA-MRSA, was found in 5.2% of the isolates. Three isolates could not be typed (Table 4). SCC*mec* type IV was found in 9% of the isolates taken in the first 48 h of hospital admission and in 4% of the isolates taken after the second day of hospital admission ($P > 0.05$).

Five isolates (5/725; 0.7%) were CA-MRSA when only the epidemiological definition was used, but three of them were SCC*mec* type III. Two isolates (2/270; 0.7%) were defined as CA-MRSA both by epidemiological features and SCC*mec* typing. On the other hand, 12 of 14 SCC*mec* type IV isolates were not defined as CA-MRSA by epidemiological features.

The only significant differences in the characteristics of the patients with SCC*mec* type IV and SCC*mec* type I–III isolates were as