



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



Devrim Dündar^{a,*}, Ayse Willke^a, Murat Sayan^{a,b}, Meliha Meric Koc^a, Ozay Arıkan Akan^c, Bulent Sumerkan^d, Nese Saltoglu^e, Akgun Yaman^f, Celal Ayaz^g, Iftihar Koksali^h

^a Kocaeli University, Kocaeli, Turkey

^b Research Center of Experimental Health Sciences, Near East University, Nicosia, North Cyprus, Turkey

^c Ankara University, Ankara, Turkey

^d Acibadem Hospital, Kayseri, Turkey

^e Istanbul University, Istanbul, Turkey

^f Çukurova University, Adana, Turkey

^g Dicle University, Diyarbakır, Turkey

^h Karadeniz Technical University, Trabzon, Turkey

ARTICLE INFO

Article history:

Received 9 April 2015

Received in revised form 18 January 2016

Accepted 5 February 2016

Available online 4 April 2016

Keywords:

MRSA

Epidemiology

Molecular characteristics

ABSTRACT

The aim of this study was to investigate the epidemiological and molecular features of clinical methicillin-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. © 2016 International Society for Chemotherapy of Infection and Cancer. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Methicillin-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

* Corresponding author. Present address: Kocaeli University, Medical Faculty, Department of Medical Microbiology, Kocaeli, Turkey. Tel.: +90 2623037443; fax: +90 2623037003.

E-mail address: devrimdundar@hotmail.com (D. Dündar).

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 methicillin resistance is located on a mobile genetic element designated *SCCmec*. This element also contains cassette chromosome recombinase (*ccr*) genes [5]. Eleven *SCCmec* types have been described to date [6]. CA-MRSA usually carries *SCCmec* types IV or V, whereas HA-MRSA usually carries *SCCmec* 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

SCCmec 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 *SCCmec* 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: *spaF1*, GAC GAT CCT TCG GTG AGC-3 (nucleotides 1096–1113); and *spaR1*, 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 \pm 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 *SCCmec* type IV isolates than in *SCCmec* type I–III isolates ($P < 0.05$) (Table 3).

The most frequent *SCCmec* type among the MRSA isolates was *SCCmec* III (91.1%). *SCCmec* type IV, which is known as the most prevalent *SCCmec* type in CA-MRSA, was found in 5.2% of the isolates. Three isolates could not be typed (Table 4). *SCCmec* 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 *SCCmec* type III. Two isolates (2/270; 0.7%) were defined as CA-MRSA both by epidemiological features and *SCCmec* typing. On the other hand, 12 of 14 *SCCmec* type IV isolates were not defined as CA-MRSA by epidemiological features.

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

Table 1

Baseline information of the enrolled patients (N = 725).

| Characteristic | n (%) ^a |
|---|--------------------|
| Age (years) (mean ± S.D.) | 52.06 ± 18.84 |
| Sex | |
| Male | 480 (66.2) |
| Female | 245 (33.8) |
| Underlying diseases | |
| Diabetes mellitus | 140 (19.3) |
| Cardiovascular disease | 114 (15.7) |
| Hypertension | 198 (27.3) |
| COPD | 59 (8.1) |
| Chronic skin disease | 14 (1.9) |
| Renal insufficiency | 100 (13.8) |
| Dialysis | 68 (9.4) |
| Alcohol abuse | 39 (5.4) |
| Smoking abuse | 147 (20.3) |
| Drug abuse | 1 (0.1) |
| HIV positivity | 1 (0.1) |
| Infection site | |
| Respiratory tract | 110 (15.2) |
| Blood and central venous catheter | 181 (25.0) |
| Urinary tract | 27 (3.7) |
| Skin and soft tissue | 120 (16.6) |
| Sterile body fluid | 20 (2.8) |
| Other | 267 (36.8) |
| Hospitalisation in the last 2 years | 383 (52.8) |
| Surgical operation in the last 2 years | 372 (51.3) |
| Use of antibiotics in the last year | 455 (62.8) |
| Hospital staff in the family | 42 (5.8) |
| Previous MRSA positivity | 85 (11.7) |
| Presence of | |
| Vascular catheter | 265 (36.6) |
| Central venous catheter | 75 (10.3) |
| Urinary catheter | 348 (48.0) |
| Tracheostomy tube | 168 (23.2) |
| Thorax tube | 16 (2.2) |
| Nasogastric tube | 43 (5.9) |
| Drainage catheter | 20 (2.8) |
| Dialysis catheter | 7 (1.0) |
| Ventilator | 21 (2.9) |
| Colostomy/cystostomy catheter | 14 (1.9) |
| Sample date according to hospital admission | |
| ≤48 h | 127 (17.5) |
| >48 h | 598 (82.5) |

S.D., standard deviation; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; MRSA, methicillin-resistant *Staphylococcus aureus*.^a Data are n (%) unless otherwise stated.**Table 3**

Comparison of antimicrobial resistance rates (%) between SCCmec type IV and SCCmec type I–III isolates.

| Antimicrobial agent | SCCmec IV (n = 14) | SCCmec type I–III (n = 253) | P-value |
|---------------------|--------------------|-----------------------------|---------|
| Ciprofloxacin | 7 (50) | 246 (97) | <0.01* |
| Gentamicin | 6 (43) | 215 (85) | 0.01* |
| Clindamycin | 2 (14) | 108 (43) | 0.07 |
| Erythromycin | 4 (29) | 151 (60) | 0.046* |
| Rifampicin | 6 (43) | 248 (98) | <0.01* |
| SXT | 0 (0) | 2 (1) | 1.000 |
| Tetracycline | 7 (50) | 244 (96) | <0.01* |
| MLSBi | 2 (14) | 40 (16) | 1.000 |

SCCmec, staphylococcal chromosome *mec*; SXT, trimethoprim/sulfamethoxazole; MLSBi, inducible clindamycin resistance.* Statistically significant difference ($P < 0.05$).

follows: patients had a tracheostomy tube in 40% of the SCCmec type IV isolates versus 89% of the SCCmec type I–III isolates ($P = 0.018$); similarly, 50% of the patients with SCCmec type IV isolates and 98% of the patients with SCCmec type I–III isolates had a urinary catheter ($P < 0.01$).

A total of 17 *spa* types were found. The most frequent *spa* type was t030 (81.1%). Twenty-three isolates (8.5%) could not be typed (Table 5). *spa* type t030 was detected in 75% of the SCCmec type I–III isolates and in 86% of the SCCmec type IV isolates. There was no significant relationship between *spa* type and SCCmec IV positivity. The relationship between *spa* and SCCmec type are shown in Table 6. A total of 201 (74.4%) of the 270 isolates were the t030–SCCmec III pattern (Table 6).

4. Discussion

Although several studies have been conducted on the molecular epidemiology of MRSA in Turkey, this is the most comprehensive multicentre study in Turkey to investigate CA-MRSA using both epidemiological and genotypic features. Among the clinical MRSA isolates from Turkey, the most common SCCmec type was SCCmec III, the most common *spa* type was t030, and the CA-MRSA rate was low (0.7%).

The general characteristics of the patients enrolled the study are shown in Table 1. Although at the beginning of the study the aim was to compare the features of CA-MRSA and HA-MRSA

Table 2Resistance rates of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates to non-β-lactam antimicrobials and distribution in different centres.

| Antimicrobial agent | Resistant isolates (N) | | | | | | Total resistant (N = 725) [n (%)] |
|---------------------|-------------------------|------------------|---------------------------|---------------------------|-------------------|----------------------------|-----------------------------------|
| | Adana Çukurova (n = 66) | Ankara (n = 295) | Diyarbakır Dicle (n = 46) | Kayseri Erciyes (n = 113) | Kocaeli (n = 160) | Trabzon Karadeniz (n = 45) | |
| CIP | 61 | 276 | 46 | 110 | 147 | 37 | 677 (93.4) |
| GEN | 51 | 247 | 43 | 94 | 120 | 34 | 589 (81.2) |
| CLI | 15 | 111 | 9 | 80 | 43 | 21 | 279 (38.5) |
| ERY | 18 | 174 | 20 | 84 | 92 | 31 | 419 (57.8) |
| FA | 0 | 1 | 0 | 1 | 0 | 0 | 2 (0.3) |
| LZD | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q/D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RIF | 62 | 279 | 46 | 111 | 145 | 38 | 681 (93.9) |
| SXT | 0 | 7 | 0 | 0 | 0 | 1 | 8 (1.1) |
| TET | 59 | 276 | 46 | 111 | 145 | 38 | 675 (93.1) |
| TEIC | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| VAN | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MUP | 0 | 1 | 1 | 0 | 0 | 0 | 2 (0.3) |
| MLSBi | 3 | 60 | 11 | 5 | 50 | 8 | 137 (18.9) |

CIP, ciprofloxacin; GEN, gentamicin; CLI, clindamycin; ERY, erythromycin; FA, fusidic acid; LZD, linezolid; Q/D, quinupristin/dalfopristin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; TEIC, teicoplanin; VAN, vancomycin; MUP, mupirocin; MLSBi, inducible clindamycin resistance.

Table 4Staphylococcal chromosome *mec* (SCC*mec*) types of 270 randomly selected meticillin-resistant *Staphylococcus aureus* (MRSA) isolates and distribution in different centres.

| SCC <i>mec</i> type | n (%) | | | | | | Total [n (%)] |
|---------------------|----------------|------------|------------------|-----------------|-----------|-------------------|---------------|
| | Adana Çukurova | Ankara | Diyarbakır Dicle | Kayseri Erciyes | Kocaeli | Trabzon Karadeniz | |
| I | – | 1 (0.9) | – | – | 1 (1.9) | 1 (5.9) | 3 (1.1) |
| II | 1 (4.2) | 3 (2.7) | – | – | – | – | 4 (1.5) |
| III | 21 (87.5) | 102 (91.1) | 20 (95.2) | 41 (97.6) | 48 (88.9) | 14 (82.4) | 246 (91.1) |
| IVa | 2 (8.3) | 3 (2.7) | 1 (4.8) | 1 (2.4) | 5 (9.3) | 2 (11.8) | 14 (5.2) |
| NT | – | 3 (2.7) | – | – | – | – | 3 (1.1) |
| Total | 24 | 112 | 21 | 42 | 54 | 17 | 270 |

NT, non-typeable.

Table 5Staphylococcal protein A (*spa*) types of 270 randomly selected meticillin-resistant *Staphylococcus aureus* (MRSA) isolates and distribution in different centres.

| <i>spa</i> type | n | | | | | | Total [n (%)] |
|-----------------|----------------|--------|------------------|-----------------|---------|-------------------|---------------|
| | Adana Çukurova | Ankara | Diyarbakır Dicle | Kayseri Erciyes | Kocaeli | Trabzon Karadeniz | |
| t030 | 18 | 83 | 21 | 37 | 47 | 13 | 219 (81.1) |
| t233 | – | 2 | – | – | – | – | 2 (0.7) |
| t459 | 2 | 1 | – | 1 | – | 2 | 6 (2.2) |
| t5168 | – | 2 | – | – | – | – | 2 (0.7) |
| t632 | 1 | 2 | – | – | – | 2 | 5 (1.9) |
| t267 | 1 | – | – | – | 1 | – | 2 (0.7) |
| t08 | – | 1 | – | – | – | – | 1 (0.4) |
| t637 | – | – | – | – | 1 | – | 1 (0.4) |
| t692 | – | – | – | – | 1 | – | 1 (0.4) |
| t2019 | 1 | – | – | – | – | – | 1 (0.4) |
| t1192 | – | 1 | – | – | – | – | 1 (0.4) |
| t1965 | – | 1 | – | – | – | – | 1 (0.4) |
| t129 | – | – | – | – | 1 | – | 1 (0.4) |
| t600 | – | 1 | – | – | – | – | 1 (0.4) |
| t1082 | – | – | – | – | 1 | – | 1 (0.4) |
| t12405 | – | – | – | – | 1 | – | 1 (0.4) |
| t12238 | – | 1 | – | – | – | – | 1 (0.4) |
| NT | 1 | 17 | – | 4 | 1 | – | 23 (8.5) |
| Total | 24 | 112 | 21 | 42 | 54 | 17 | 270 |

NT, non-typeable.

isolates, this could not be performed because of the small number of CA-MRSA isolates.

High resistance rates among the MRSA isolates to ciprofloxacin, gentamicin, rifampicin and tetracycline were found in this study (Table 2). This finding may be related to the irrational use of antimicrobials in hospitals and community settings and the presence of one dominating clone expressing this resistance phenotype. It was also found that SCC*mec* type IV isolates were more susceptible to most non-β-lactam antimicrobials compared with the other SCC*mec* types (Table 3). Although clindamycin resistance was also lower in SCC*mec* type IV isolates than the other SCC*mec* types, the difference was not statistically significant ($P = 0.07$) because the expected count was <5 . The SCC*mec* type IV element is smaller than the other SCC*mec* types and generally does not contain any additional resistance genes. Therefore, SCC*mec* type IV isolates are more susceptible to non-β-lactam antimicrobials than the other SCC*mec* types [11,12].

According to the current results, SCC*mec* type III was found to be the most common SCC*mec* type (Table 4), similar to the results of previous studies from Turkey (82–100%) [12–15]. According to the current data, SCC*mec* type IV isolates, known as the main SCC*mec* type in CA-MRSA, comprised 5.2% of the MRSA isolates. SCC*mec* type IV positivity has been reported to be 2.6–7.6% from Turkey [12,14,15].

No statistically significant difference was found in SCC*mec* type IV positivity rates between MRSA isolates from the samples taken within 2 days after admission and after the second day. This shows that the date of sampling alone is not enough for CA-MRSA

definition. In this study, three of the five isolates that were epidemiologically defined as CA-MRSA were found to contain SCC*mec* type III, and only 2 of the 14 SCC*mec* type IV isolates were defined as CA-MRSA by epidemiological features. In a previous study, SCC*mec* typing of HA-MRSA isolates revealed that 13.1% of strains carried SCC*mec* type V, which is mainly carried by CA-MRSA isolates, indicating that HA-MRSA and CA-MRSA isolates might not be distinguished merely by identifying their SCC*mec* genotypes [16]. In fact, using SCC*mec* typing as a marker for CA-MRSA does not appear to be suitable because isolates with a non-typeable SCC*mec* may be missed, and SCC*mec* type IV-carrying HA-MRSA lineages may be misclassified as CA-MRSA [1]. It is also known that only epidemiological or only genotypic definition is inadequate for diagnosis of CA-MRSA [1].

We determined higher rates of tracheostomy tube and urinary catheter usage in SCC*mec* type I–III MRSA-carrying patients than SCC*mec* type IV MRSA-carrying patients. This may be explained by the fact that use of these devices is more common among hospitalised patients and SCC*mec* type III is the most frequent type.

In the present study, the most common *spa* type was t030 (Table 5) and there was no statistically significant relationship between SCC*mec* type IV positivity and *spa* type. t030 has been found to be the most common *spa* type (85–98%) in previous studies from Turkey [13,14,17]. Bozdoğan et al. reported that the most common *spa* type was t030 in SCC*mec* type III isolates but t005 in SCC*mec* type IV isolates [14]. MRSA *spa* types have a predominantly regional distribution in the world. t030, t037 and t002 are the predominant *spa* types in HA-MRSA isolates in China

Table 6

Relationship between staphylococcal protein A (*spa*) and staphylococcal chromosome *mec* (SCC*mec*) types of 270 randomly selected methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

| <i>spa</i> /SCC <i>mec</i> pattern | | Centre | | | | | | Total [n (%)] |
|------------------------------------|---------------------|----------------|-----------------|------------------|-----------------|-----------------|-------------------|---------------|
| <i>spa</i> type | SCC <i>mec</i> type | Adana Çukurova | Ankara | Diyarbakır Dicle | Kayseri Erciyes | Kocaeli | Trabzon Karadeniz | |
| t030 | III | 16 | 77 ^a | 20 | 36 | 41 ^b | 11 | 201 (74.4) |
| t030 | I | – | 1 | – | – | 1 | – | 2 (0.7) |
| t030 | NT | – | 2 | – | – | – | – | 2 (0.7) |
| t030 | IV | 1 | – | 1 | 1 | 5 ^b | 2 | 10 (3.7) |
| t030 | II | 1 | 3 | – | – | – | – | 4 (1.5) |
| t233 | III | – | 2 | – | – | – | – | 2 (0.7) |
| t459 | III | 2 | 1 | – | 1 | – | 2 | 6 (2.2) |
| t5168 | IV | – | 1 ^b | – | – | – | – | 1 (0.4) |
| t5168 | NT | – | 1 | – | – | – | – | 1 (0.4) |
| t632 | III | 1 | 2 | – | – | – | 1 | 4 (1.5) |
| t632 | I | – | – | – | – | – | 1 | 1 (0.4) |
| t267 | III | 1 | – | – | – | 1 | – | 2 (0.7) |
| t08 | IV | – | 1 | – | – | – | – | 1 (0.4) |
| t637 | III | – | – | – | – | 1 | – | 1 (0.4) |
| t692 | III | – | – | – | – | 1 | – | 1 (0.4) |
| t2019 | III | 1 | – | – | – | – | – | 1 (0.4) |
| t1192 | III | – | 1 | – | – | – | – | 1 (0.4) |
| t1965 | III | – | 1 | – | – | – | – | 1 (0.4) |
| t129 | III | – | – | – | – | 1 | – | 1 (0.4) |
| t600 | III | – | 1 | – | – | – | – | 1 (0.4) |
| t1082 | III | – | – | – | – | 1 | – | 1 (0.4) |
| t12238 | III | – | 1 | – | – | – | – | 1 (0.4) |
| t12405 | III | – | – | – | – | 1 | – | 1 (0.4) |
| NT | III | – | 16 | – | 4 | 1 | – | 21 (7.8) |
| NT | IV | 1 | 1 | – | – | – | – | 2 (0.7) |
| Total | | 24 | 112 | 21 | 42 | 54 | 17 | 270 |

NT, non-typeable.

^a Two isolates were epidemiologically defined as CA-MRSA.

^b One isolate was epidemiologically defined as CA-MRSA.

[18]. The major *spa* types are t437, t019 and t324 among CA-MRSA isolates and t037, t002 and t425 among HA-MRSA isolates in Asian countries [19]. In Europe, the most prevalent *spa* type is t032 followed by t003. However, the distribution of *spa* types varies in European countries. For example, in analogy with our results, t030 is predominant in Bulgaria and Romania, which are close to Turkey [20]. On the other hand, although we performed *spa* typing twice, the number of non-typeable isolates in the *spa* analysis was higher than expected (23/270; 8.5%) in this study. Since the PCR products from untyped isolates were well sequenced, non-typeability may be associated with geographical differences and/or an unknown *spa* type.

spa typing is an easy, rapid and portable method and has standard nomenclature. However, it is insufficiently discriminatory in regions where a particular clone is endemic. It is not recommended for small local hospital laboratories [4]. It has been shown that *spa* typing used for classifying MRSA as belonging to either a community or hospital clone is of limited value to indicate the setting where MRSA was actually acquired [7]. Tang et al. showed that 20 strains with the same *spa* type collected during an outbreak exhibited several related but distinguishable PFGE patterns [21]. Moreover, as a result of mutations affecting the *spa* gene, isolates that belong to different *spa* types may in fact be closely related [22].

The predominance of *spa* type t030-SCC*mec* III isolates suggested that the major clone is ST239-MRSA-III in Turkey, as reported previously [14,17]. This is known as the Brazilian/Hungarian clone and is distributed differently in many regions of the world (Asia, Australia, South Africa, South America and Europe) [4].

The data in this study show that the prevalence of CA-MRSA is low in Turkey (0.7%). In a previous study, this rate was reported as 7% [13]. The incidence of CA-MRSA varies among countries, from 2.9% in Spain [23], to 27% in Austria [7], 28.9% in Canada [24] and

46% in the USA [25]. In a study conducted in eight Asian countries, the proportion of CA-MRSA as a percentage of total MRSA varied from <5% to >30% [19].

Recent studies have shown evidence of CA-MRSA spreading in healthcare settings and, in recent years, healthcare-associated infections caused by CA-MRSA has been reported [1,4,26]. Song et al. reported that MRSA clones have spread between the community and hospitals as well as between countries. CA-MRSA isolates spread from the community to hospitals as well as HA-MRSA spreading to the community [19]. Traditional distinctions between HA-MRSA and CA-MRSA based on clinical epidemiology and susceptibility are becoming increasingly less relevant [6]. Repeatedly, CA-MRSA episodes are increasingly likely to be misclassified as HA-MRSA by epidemiological definitions [1]. Epidemiological definitions are further limited by the emergence of CA-MRSA clones as an increasingly common cause of healthcare-associated infection. Purely epidemiological definitions will misclassify CA-MRSA acquired in hospital [1]. It is suggested that combining epidemiological definition with a genotyping method such as MLST, SCC*mec* analysis, *spa* typing and PFGE is the best diagnostic approach for CA-MRSA [1]. The findings of this study also support this view.

As a limitation of this study, comparisons between CA-MRSA and HA-MRSA could not be made due to the low prevalence of CA-MRSA.

In conclusion, the epidemiology of MRSA throughout the world is changing rapidly and the definition of CA-MRSA is becoming more complicated. Molecular typing methods are not sufficient alone. Therefore, combined use of epidemiological and genotypic methods is the most accurate approach for the diagnosis of CA-MRSA.

Funding

This work was supported by the Scientific Research Unit of Kocaeli University (Kocaeli, Turkey) [2005/3].

Competing interests

None declared.

Ethical approval

This study was approved by the Ethics Committee of Kocaeli University Faculty of Medicine [2005/46].

Acknowledgments

The authors would like to thank to Prof. Dr. Onur Hamzaoglu and Prof. Dr. Canan Baydemir for their support in the statistical analysis.

References

- [1] Otter JA, French GL. Community-associated methicillin-resistant *Staphylococcus aureus*: the case for a genotypic definition. *J Hosp Infect* 2012;81:143–8.
- [2] Otto M. Community-associated MRSA: what makes them special? *Int J Med Microbiol* 2013;303:324–30.
- [3] US Centers for Disease Control and Prevention (CDC). Methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Atlanta, GA: CDC, <http://www.cdc.gov/mrsa/index.html> [accessed 17.07.12].
- [4] Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 2012;39:273–82.
- [5] Francois P, Renzi G, Pittet D, Bento M, Lew D, Harbarth S, et al. A novel multiplex real-time PCR assay for rapid typing of major staphylococcal cassette chromosome mec elements. *J Clin Microbiol* 2004;42:3309–12.
- [6] Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Curr Opin Microbiol* 2012;15:588–95.
- [7] Schmid D, Simons E, Ruppitsch W, Hrivniaková L, Stoeger A, Wechsler-Fördös A, et al. Limited value of routine *spa* typing: a cross-sectional study of methicillin-resistant *Staphylococcus aureus*-positive patients in an Austrian hospital. *Am J Infect Control* 2013;41:617–24.
- [8] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. Document M100-S20. Wayne, PA: CLSI; 2010.
- [9] Shopsis B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999;37:3556–63.
- [10] Oliveira DC, Crisóstomo I, Santos-Sanches I, Major P, Alves CR, Aires-de-Sousa M, et al. Comparison of DNA sequencing of the protein A gene polymorphic region with other molecular typing techniques for typing two epidemiologically diverse collections of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2001;39:574–80.
- [11] Maltezou HC, Giamarellou H. Community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Int J Antimicrob Agents* 2006;27:87–96.
- [12] Karahan ZC, Tekeli A, Adaleti R, Koyuncu E, Dolapci I, Akan OA. Investigation of Pantone–Valentine leukocidin genes and SCCmec types in clinical *Staphylococcus aureus* isolates from Turkey. *Microb Drug Resist* 2008;14:203–10.
- [13] Alp E, Klaassen CH, Doganay M, Altöparlak U, Aydın K, Engin A, et al. MRSA genotypes in Turkey: persistence over 10 years of a single clone of ST239. *J Infect* 2009;58:433–8.
- [14] Bozdoğan B, Yıldız O, Oryaşın E, Kırdar S, Gülcü B, Aktepe O, et al. t030 is the most common *spa* type among methicillin-resistant *Staphylococcus aureus* strains isolated from Turkish hospitals. *Mikrobiyol Bul* 2013;47:571–81 [in Turkish].
- [15] Kilic A, Guclu AU, Senses Z, Bedir O, Aydoğan H, Basustaoglu AC. Staphylococcal cassette chromosome mec (SCCmec) characterization and Pantone–Valentine leukocidin gene occurrence for methicillin-resistant *Staphylococcus aureus* in Turkey, from 2003 to 2006. *Antonie Van Leeuwenhoek* 2008;94:607–14.
- [16] Sun DD, Ma XX, Hu J, Tian Y, Pang L, Shang H, et al. Epidemiological and molecular characterization of community and hospital acquired *Staphylococcus aureus* strains prevailing in Shenyang, Northeastern China. *Braz J Infect Dis* 2013;17:682–90.
- [17] Kırca Yılmaz S, Acuner IC, Strommenger B, Bek Y, Witte W. Infectivity–resistotype–genotype clustering of methicillin-resistant *Staphylococcus aureus* strains in the Central Blacksea Region of Turkey. *Mikrobiyol Bul* 2014;48:14–27 [in Turkish].
- [18] Xiao M, Wang H, Zhao Y, Mao LL, Brown M, Yu YS, et al. National surveillance of methicillin-resistant *Staphylococcus aureus* in China highlights a still-evolving epidemiology with 15 novel emerging multilocus sequence types. *J Clin Microbiol* 2013;51:3638–44.
- [19] Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *J Antimicrob Chemother* 2011;66:1061–9.
- [20] Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, et al. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular–epidemiological analysis. *PLoS Med* 2010;7:e1000215.
- [21] Tang YW, Waddington MG, Smith DH, Manahan JM, Kohner PC, Highsmith LM, et al. Comparison of protein A gene sequencing with pulsed-field gel electrophoresis and epidemiologic data for molecular typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2000;38:1347–51.
- [22] Price J, Gordon NC, Crook D, Llewelyn M, Paul J. The usefulness of whole genome sequencing in the management of *Staphylococcus aureus* infections. *Clin Microbiol Infect* 2013;19:784–9.
- [23] Vindel A, Trincado P, Cuevas O, Ballesteros C, Bouza E, Cercenado E. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Spain: 2004–12. *J Antimicrob Chemother* 2014;69:2913–9.
- [24] Nichol KA, Adam HJ, Roscoe DL, Golding GR, Lagacé-Wiens PR, Hoban DJ, et al. Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Canada. *J Antimicrob Chemother* 2013;68(Suppl. 1):i47–55.
- [25] Murphy CR, Hudson LO, Spratt BG, Elkins K, Terpstra L, Gombosev A, et al. Predictors of hospitals with endemic community-associated methicillin-resistant *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 2013;34:581–7.
- [26] Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing health-care-associated infections. *Emerg Infect Dis* 2007;13:236–42.