Molecular and Epidemiological Evaluation of Strain Replacement in Patients Previously Harboring Gentamicin-Resistant MRSA

Giulia De Angelis, Patrice Francois, Andie Lee, Jacques Schrenzel, Gesuele Renzi, Myriam Girard, Didier Pittet, and Stephan Harbarth

Division of Infectious Diseases, Università Cattolica Sacro Cuore, Rome, Italy; Genomic Research Laboratory, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland; Department of Infectious Diseases and Microbiology, Royal Prince Alfred Hospital, Sydney, Australia; and Infection Control Program, University of Geneva Hospitals, and Faculty of Medicine, Geneva, Switzerland

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Gentamicin-susceptible methicillin-resistant Staphylococcus aureus (GS-MRSA) clones have gradually replaced gentamicin-resistant MRSA (GR-MRSA) clones in many European countries. We studied molecular and epidemiological aspects of MRSA strain replacement in individual patients. All patients from whom at least 2 MRSA strains showing different gentamicin susceptibility patterns were isolated between 1996 and 2008 were retrospectively identified. Staphylococcal cassette chromosome mec (SCCmec) type and clonality between isolates were determined using molecular methods. Risk factors for individual GR-MRSA SCCmec I (prevalent clone) strain replacement with GS-MRSA non-SCCmec I types were studied in a nested case-crossover study (n = 55 patients). MRSA strain replacement was observed in 127 patients, 85 (67%) of whom were initially colonized with GR-MRSA replaced subsequently by GS-MRSA. Most GS-MRSA replacement strains (50; 59%) possessed SCCmec IV. All MRSA isolate pairs from the same patient that consisted of different gentamicin susceptibility and SCCmec types were also genotypically different. Exposure to domiciliary nursing assistance (odds ratio [OR], 8.1; 95% confidence interval [CI], 1.2 to 53.7) and high Charlson scores (OR, 7.1; 95% CI, 1.1 to 46.8) were associated with individual strain replacement. In individual patients, exogenous acquisition of a different MRSA strain was responsible for strain replacement in most cases. Domiciliary nursing assistance could be a target for specific control measures to prevent transmission of GS-MRSA in our setting.

Since its first appearance in 1961, the epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) has changed over time and continues to rapidly evolve (2, 13). The genetic basis of methicillin resistance in S. aureus is the presence of mecA, which is part of the staphylococcal cassette chromosome mec (SCCmec) located on a mobile genetic element inserted in orfX (12). To date, eight differently organized SCCmec elements have been characterized (11). New MRSA clones regularly emerge, sometimes replacing previously predominant clones. Clonal shift has been observed in several countries, in small regions within a country, or in single hospitals (5, 6, 20). However, there are limited data regarding the epidemiology and potential risk factors for the replacement of endemic strains with new MRSA clones in a given hospital and at the level of individual patients. Most previous studies have been conducted using ecologic data only or generated molecular data of limited clinical interest (1).

At the University of Geneva Hospitals (HUG), the incidence of MRSA increased until 2005 and then substantially decreased (15, 22). The endemic health care-associated (HA) MRSA strain at HUG is sequence type (ST) 228-SCCmec I (the Southern German clone), which is resistant to gentamicin, ciprofloxacin, clindamycin, and erythromycin. The genetic basis of aminoglycoside resistance in MRSA is related to the acquisition of genes encoding aminoglycoside-modifying enzymes (18). The resistance determinant is generally not part of the SCCmec element but belongs to other mobile elements, such as plasmids (e.g., Mu50 or JH1 and JH9) or transposons (e.g., Tn4001 in TW20). The prevalence of gentamicin-susceptible MRSA (GS-MRSA) has gradually increased over the last 5 years at our institution, while a parallel decrease of gentamicin-resistant MRSA (GR-MRSA) isolates was observed, indicating possible clonal replacement. Indeed, during the last 10 years, constant importation of the HA-MRSA strain ST8-SCCmec IV from neighboring France into the Geneva area has been observed (7), and this has been associated with sporadic nosocomial infections. This clone, heteroresistant to methicillin and susceptible to gentamicin, had previously replaced the Iberian clone (ST247-SCCmec I) in French hospitals, reaching up to 70% of all MRSA isolates in 2007 (6). A similar increase in isolates harboring the type IV SCCmec element has been observed in the university hospital in Basel, Switzerland (21).

The present study retrospectively evaluated the frequency, molecular patterns, and main epidemiological factors associated with MRSA phenotype replacement in individual patients at our institution, using a case-crossover study and molecular investigations.
RESULTS

Descriptive epidemiology. Over the study period of 1996 to 2008, 127 individual patients had at least one replacement of MRSA strains based on a change in gentamicin susceptibility of the isolates. Forty-one (32%) patients showed more than one (up to four) MRSA strain shifts over time. Among 289 strains included and tested from these 127 patients, SCCmec I and IV were the most frequently identified cassettes in GR-MRSA (133 strains; 92%) and GS-MRSA (99 strains; 69%), respectively.

Eighty-five patients (67%) had GR-MRSA at baseline, followed by replacement with GS-MRSA after a median of 71 weeks (range, 0 to 504 weeks). SCCmec I was detected in 95% of these GR-MRSA baseline strains. SCCmec IV was detected in 50 (59%) of GS-MRSA replacement strains, of which 78% (39/50) were identified over the last 5 years. A switch from GR-MRSA SCCmec I to GS-MRSA SCCmec IV in the same patient was the strain replacement most frequently observed (47 strain replacements; 55%), followed by the conservation of the same cassette despite a change in gentamicin susceptibility pattern (19 strain replacements; 22%). Of note, 17 pairs conserved the SCCmec type I. Replacement with strains harboring SCCmec II (8), III (2), or V (6) was uncommon.

Sixty-one patients were eligible for inclusion in the risk factor analysis. Six patients were excluded because the time between baseline and replacement strain isolation was less than 1 month. The final population included in the case-crossover study consisted of 55 patients, whose baseline GR-MRSA strains harboring SCCmec I were followed by the isolation of GS-MRSA harboring SCCmec types other than type I: 41 of them showed strain replacement with GS-MRSA SCCmec IV, 7 with GS-MRSA SCCmec II, 5 with GS-MRSA SCCmec V, and 2 with GS-MRSA SCCmec III.

Molecular analysis. In the MLVA of the 289 strains from the 127 patients, SCCmec IV MRSA strains were segregated in approximately 10 profiles showing distance >0.2 (8) and were mainly strains belonging to clonal complex 8 (CC8). The most homogeneous and largest cluster contained isolates of the prevalent SCCmec I ST228 clone belonging to CC8. The remaining MRSA SCCmec IV replacement strains were identified as ST995 (11 strains; 26%), ST5 (2 strains), ST1 (3 strains), ST80 (1 strain), ST88 (1 strain), and ST30 (1 strain).

Eighty-six percent (16 of 19) of MRSA pairs from the same patient which possessed the same SCCmec type despite a change in gentamicin susceptibility were clonally related. No SCCmec replacement was observed in clonally related isolates from the same patient. No genotypic correlation was found among MRSA strains expressing different cassette types.

Case-crossover study. Table 1 summarizes characteristics of case and control periods in the month prior to the isolation of baseline and replacement MRSA strains in the same patient.
As expected, several variables showed no within-group variance as they stayed constant over time (e.g., gender, professional activity, gastric ulcer, and liver disease). By univariate analysis, previous hospitalization in a Swiss hospital, domiciliary nursing assistance, presence of renal failure, previous antibiotic therapy with \( \beta \)-lactams, and a higher Charlson comorbidity index were all factors significantly associated with the shift from GR-MRSA SCC\(_{\text{mec}}\)I to GS-MRSA with a different SCC\(_{\text{mec}}\) type.

By multivariate conditional regression analysis, time periods in which cases were exposed to domiciliary nursing assistance (OR, 8.1; 95% CI, 2.9 to 23.6) and higher Charlson scores (per 1-point increment; OR, 7.1; 95% CI, 1.1 to 46.8) were associated with the isolation of GS-MRSA characterized by an SCC\(_{\text{mec}}\) nonotype I from a patient previously harboring GR-MRSA SCC\(_{\text{mec}}\)I. Prior exposure to \( \beta \)-lactams (OR, 0.09; 95% CI, 0.01 to 0.60) significantly increased the risk of isolation of GR-MRSA SCC\(_{\text{mec}}\)I during the control periods.

**DISCUSSION**

This study showed that the majority of individual replacement events of the predominant HA-MRSA South German clone in Geneva was observed in two distinct situations: the
first, and most common, was the shift to GS-MRSA harboring the staphylococcal cassette IV; the second, and less frequent but still important, was the conservation of the same cassette despite a change in phenotypic features of the replacement strain. Importantly, phenotypic change among clonally related MRSA strains isolated from the same patient was never associated with the acquisition of a different staphylococcal cassette. On the contrary, replacement strains with a different cassette type were genotypically distinct from the previously isolated strains from the same patient, supporting the hypothesis of exogenous acquisition. The epidemiologic analysis of this replacement phenomenon suggests that domiciliary nursing assistance and a worsening of comorbid conditions were associated with replacement of GR-MRSA SCC mec I strains by GS-MRSA containing a different cassette within the same patient. The role of domiciliary nursing assistance has been previously investigated in a prospective case-control study conducted in a French teaching hospital which showed that prior receipt of home nursing care was a risk factor for MRSA infection at hospital admission (16). The authors concluded that community nurses appeared to be potential vectors of MRSA among patients without prior hospitalization. Similarly, our study showed that home nursing assistance could be a risk factor for the acquisition of a GS-MRSA with a staphylococcal cassette other than type I in the same patient. The MLVA performed on the MRSA strains characterized by the cassette IV which replaced the South German clone (ST228) showed that 83% of them belonged to type CC8, among which 22 (65%) were identified as the STS French clone. Molecular analysis excluded the hypothesis that the replacing GS-MRSA SCC mec IV strains belonged to typical European community clones, such as ST80 and ST88, which were rarely isolated in our study. The proximity of the French border and the high proportion (>70%) of French nurses working in the Geneva health care setting may explain why the replacement strain isolated in our patients was commonly the STS French clone.

Prior exposure to β-lactams was a protective factor against individual replacement of GR-MRSA SCC mec I with GS-MRSA with SCC mec other than type I. A matched-pair case-case study performed in 1996 which investigated major risk factors for GS-MRSA acquisition showed that patients with GS-MRSA were less likely to have received β-lactams than patients with GR-MRSA (19). In this study, the authors postulated that this association may be due to penicillinase-resistant β-lactams having some activity against GS-MRSA, which exhibit heterogeneous resistance to methicillin but not against GR-MRSA, which is homogeneously resistant to methicillin (19).

The major limitation of our study relates to retrospective data collection which increased the risk of information bias. In particular, due to the lack of reliable data sources, outpatient antibiotic exposure was not recorded. In addition, we cannot exclude the effect of seasonality on the outcome. Neither transmission events nor exact dates of acquisition could be ascertained due to the absence of prospective, systematic MRSA screening. This information would have allowed for optimal timing of collection of risk factor data. The frequency of strain replacement may have been underestimated, as not all patients who were MRSA colonized may have been rescreened or readmitted to the hospital, particularly those with fewer co-morbidities. Finally, MLVA is characterized by a higher discriminatory power than other molecular methods, including pulsed-field gel electrophoresis and MLST (7). While this represents an advantage of the method, it is probably responsible for the high molecular heterogeneity among SCC mec IV strains belonging to CC8.

To conclude, in individual patients, the switch from GR SCC mec I MRSA to health-care-associated GS SCC mec IV MRSA strains was most frequently observed over the study period. The isolation of GS-MRSA harboring the staphylococcal cassette IV is a recent phenomenon in Geneva, and exogenous acquisition of a different MRSA strain was frequently associated with domiciliary nursing assistance. MRSA strain replacement in the individual patient may have clinical implications in terms of antibiotic treatment options and isolation policies.

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