Methicillin-resistant *Staphylococcus aureus* carriage in a long-term care facility: hypothesis about selection and transmission

MATTHIEU EVEILLARD1, PHILIPPE CHARRU2, PIERRE RUFAT3, MARIE-CLaire HIPPEAUX1, EVELYNE LANCiEN1, FARIDA BENSELAMA2, CATHERINE BRANGER1

1Hôpital Louis Mourier (AP-HP), Microbiologie-hygiène, France
2Hôpital Louis Mourier, Gérontologie, France
3Groupe Hospitalier Pitié Salpêtrière, Santé publique, France

Address correspondence to: Matthieu Eveillard. Email: maeveillard@chu-angers.fr

Abstract

**Background:** many studies have identified long-term care facilities (LTCFs) as reservoirs of patients carrying methicillin-resistant *Staphylococcus aureus* (MRSA). However, few data about the mechanisms of MRSA diffusion in these settings are available.

**Objectives:** the purpose of our study was to suggest hypothesis on the possible ways of MRSA transmission to residents in or outside a LTCF.

**Methods:** data concerning patients on the day of the survey and within the preceding year were collected. Multivariate analysis was performed by logistic regression to identify characteristics associated with MRSA carriage. MRSA strains were analysed by pulsed-field gel electrophoresis (PFGE) and the relatedness between DNA patterns was studied with Gel Compar software.

**Results:** the prevalence of MRSA carriage was 37.6%. Treatment with fluoroquinolones or third-generation cephalosporins [odds ratio (OR) = 12.07; 95% confidence interval (CI) = 5.90–24.7], treatment with other antimicrobial agents (OR = 4.40; 95% CI = 2.43–7.97), at least one medical imaging session (OR = 5.08; 95% CI = 2.66–9.69) within the 12 preceding months, and the presence of a subcutaneous catheter on the day of the survey (OR = 3.09; 95% CI = 1.87–5.10) were independently associated with MRSA carriage. Twenty-eight of the 38 strains tested were clustered in two major groups. In each of these groups, strains had at least a 90% relatedness. These strains were isolated in patients hospitalised in different areas of the LTCF.

**Conclusion:** we identified that both molecular and epidemiological arguments support the hypothesis of the possibility of MRSA cross-transmission inside the LTCF. Further studies are needed to confirm and explain the association identified between MRSA carriage and medical imaging.

**Keywords:** MRSA carriage, long-term care facility, cross-transmission, elderly

Introduction

It is well established that for the elderly, institutionalisation in a nursing home (NH) or a long-term care facility (LTCF) is a risk factor of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. During the past decade, several reports of high occurrences of MRSA colonisation in NH or LTCF have been reported. Indeed, some US facilities have reported rates of colonisation as high as 30% [1]. In a recent study [2] conducted in the emergency department of a French teaching hospital, the prevalence of MRSA carriage at the time of admission in patients coming from NH and LTCF was 31.9%, underscoring the high proportion of carriers in these settings. However, few data concerning cross-transmission in such facilities are available.

Our objective was to identify and quantify the reservoir of MRSA constituted by patients hospitalised in our LTCF, and to suggest hypothesis on the ways of MRSA transmission by residents inside the LTCF and when they leave this setting for different reasons (e.g. acute-care hospitalisation, medical imaging, physical therapy). For this purpose, we identified characteristics associated with carriage, and compared strains isolated by molecular typing.
MRSA carriage in a long-term care facility

Methods

Study design
In February 2004, a MRSA-carriage prevalence study was performed in a 120-bed LTCF containing three units (A, B, C) and belonging to a French teaching hospital. The mean length of stay in this setting is >1 year. Patients newly admitted to the LTCF arrived from the community. However, they could have been hospitalised in some acute-care wards within the preceding year. This facility is located in a building separated from the main building with acute-care facilities. All patients hospitalised during this period in the LTCF were sampled (anterior nares and rectal swabs) for MRSA screening. A urine culture was also systematically performed for patients with a urinary-tract catheter. Lastly, all skin wounds or lesions were sampled.

Data collection
For all sampled patients, data concerning the patients on the day of the screening [gender, age, GIR (Groupes Iso-Ressources) score [3], invasive procedures, open wounds, bedsores, or chronic skin lesions] and data concerning the patient’s background within the last 12 months (antimicrobial therapy, surgical interventions, endoscopic procedures, medical imaging, physical therapy) were recorded. The French AGGIR (Autonomie Gérontologie Groupes Iso-Ressources) model relies on the assessment of eight measures of disability and two additional measures of intellectual coherence and orientation. The model results in a classification of six GIR scores [3]. Scores <3 correspond to severe disabilities. Concerning antimicrobial therapy, the names of antimicrobial agents and the duration of each antibiotic course were noted. Concerning medical imaging [radiography, echography or computed tomography scan (RECTS)], the number of examinations was recorded for each patient included in the study. All these data were assessed by medical record review and using a standard questionnaire.

Microbiology
For isolation of single colonies, specimens were streaked on mannitol salt agar (Chapman medium, bioMérieux, Marcy l’Etoile, France) containing 5 mg/l of cefoxitin and incubated at 37°C. Plates were examined for staphylococci after 24 and 48 h. Colonies recovered were tested for coagulase. Any coagulase-positive isolate was tested for susceptibility to antimicrobial agents by the disk-diffusion method in Muller-Hinton agar according to the recommendations of the Antibiotic Committee of the French Society for Microbiology to confirm methicillin-resistance (http://www.sfim.asso.fr). Strain conservation was performed by freezing at –80°C with glycerol.

Molecular typing
MRSA isolates were typed using pulsed-field gel electrophoresis (PFGE). DNA fragment patterns were obtained as described previously [4] after digestion of genomic DNA with the restriction endonuclease Smal and subsequent PFGE. The DNA patterns obtained by PFGE were analysed with Gel Compar software (Applied Maths, Kortrijk, Belgium). A similarity index was determined for each pair of isolates by the Dice coefficient and was used to estimate the degree of inter-isolate relatedness. Clustering correlation coefficients were calculated by the unweighted pair group method with arithmetic averages (UPGMA) determined by using the band-based Dice similarity coefficient. Isolates were considered closely related when their patterns differed by three or fewer bands [5].

Statistical analysis
Data acquisition and univariate analysis were performed with Epi-Info software (version 6.04; Centers for Disease Control, Atlanta, USA). Univariate analysis was performed using chi-square or Fisher’s exact tests for comparison of qualitative variables, and using the Kruskall–Wallis test for continuous variables. Logistic regression (SPSS PC; SPSS Inc, Chicago, IL) was used to determine variables independently associated with MRSA carriage. Qualitative variables with a P-value <0.25 in univariate analysis were included as candidate variables for multivariable model inclusion. All tests were two-tailed, and a P-value <0.05 was considered significant in the multivariate model.

Results
Nasal and rectal swabs were obtained from 109 patients. Among them, 39 were hospitalised in the unit A, 33 in the unit B, and 37 in the unit C. The characteristics (median age, gender, severity of impairment, median length of hospitalisation from admission in the LTCF up to the day of the survey) of patients were similar in those three units.

The prevalence of MRSA carriage was 37.6% (95% CI 28.5–46.7%) corresponding to 41 MRSA carriers. All those patients were only colonised (no one was infected with MRSA). Among them, 11 were identified in the unit A, 12 in the unit B and 18 in the unit C. Among the 58 MRSA isolated in the different sampled sites, 27 were recovered in rectal samples, 26 in nasal samples, 3 in urine samples from residents with indwelling urinary catheters.

Despite a theoretically appropriate conservation procedure, 8 MRSA strains could not be sub-cultured for PFGE analysis. Therefore, among the 58 MRSA isolated, only 50 strains from 34 different patients were available and analysed by PFGE. Results showed that 30 patients carried a unique strain whereas four patients carried two different strains. Therefore, the data analysis was based on these 38 PFGE patterns. Twenty PFGE patterns that differed by at least one band were identified. Four patterns were identified in more than one strain. The pattern 4c was recovered in eight strains, the pattern 4d in four strains, the pattern 7a in seven strains and the pattern 7c in three strains. The pattern 4d was exclusively identified in strains isolated in the unit B, the pattern 7a was mainly identified in strains from the unit C, and half the strains presenting the pattern 4c were isolated in the unit.
B (see Appendix 1 in the supplementary data on the journal’s website http://www.ageing.oxfordjournals.org). There was no correlation between the anatomical site (nasal or rectal) of MRSA carriage and genomic groups. In the 15 patients for whom nasal and rectal MRSA strains were analysed by PFGE, four carried two different strains. Closely related strains differing in their patterns by three bands or less were clustered in 10 genomic groups. In each group, strains had more than 90% relatedness. Genomic types were designated with a numeral and each of their variant sub-types by a letter suffix (see Appendix 1 in the supplementary data on the journal’s website http://www.ageing.oxfordjournals.org). In all, 28 out of the tested 38 strains clustered in two major groups, group 4 (14 strains) and group 7 (14 strains). The 10 remaining strains showed diversity and clustered into eight other groups. Strains belonging to the two major groups were recovered in the three units, showing the spread of these two clones in the whole LTCF.

The results of the univariate analysis are presented in Table 1. Whereas some usual risk factors of MRSA carriage like antimicrobial therapy, invasive procedures and skin lesions were significantly associated with carriage, it was not the case of recent hospitalisation in an acute ward. An antimicrobial therapy within the preceding year showed the strongest and the most significant association with MRSA carriage. However, this association varied according to antimicrobial agents (Table 2). Among patients treated with at least one antimicrobial agent, the mean cumulated duration of antibiotic courses was 21.4 days in MRSA carriers and 14.0 days in non-carriers. However, this difference was not statistically significant. Most of these antimicrobial agents were prescribed and administered during the patients’ stay in the LTCF. The results of multivariate analysis are presented in Table 3. Treatment(s) with fluoroquinolones or third-generation cephalosporins, treatment(s) with other antimicrobial agents, at least one RECTS session within the last year showed the prevalence of MRSA carriage as high as the prevalence of nasal carriage, it was not the case of recent hospitalisation in an acute ward. An antimicrobial therapy within the preceding year showed the strength of the association was particularly important for fluoroquinolone or third-generation cephalosporin treatment(s). In patients who had undergone at least one RECTS session, the prevalence of MRSA carriage was higher when the number of sessions was more than two, than when it was one or two (61.5 versus 34.3%, OR = 3.07, P < 0.02).

**Discussion**

The prevalence of MRSA carriage reported in our study is higher than rates which have been recorded in other surveys in Europe. Indeed, if some US facilities have reported rates of colonisation as high as 30% [1], more recent surveys performed in Europe (Germany [6], United Kingdom [7], Belgium [8], and Slovenia [9]) reported prevalence ranging from 1.1 to 11.8%. In our study, the prevalence of MRSA rectal carriage was as high as the prevalence of nasal carriage. Further studies would be useful to try to understand this surprising result. Indeed, numerous studies have demonstrated the preponderance of nasal carriage for MRSA whereas the rectum is usually considered as an accessory reservoir [10–12].

According to a study performed in a NH, the prescription of ciprofloxacin was appropriate in only 25% of the cases [13]. Similarly, a recent study [14] conducted in a university-affiliated hospital revealed that 30% of 1941 days of antimicrobial therapy were deemed unnecessary. This rate was 36.7% for fluoroquinolones and 65.0% for ceftriaxone. Like in our study, recent reports have demonstrated a strong and significant association between individual exposure to fluoroquinolones and MRSA carriage [15–17]. A treatment with such broad-spectrum antimicrobial agents can select naturally (third-generation cephalosporins) or usually (fluoroquinolones) resistant strains like MRSA. The absence of significant association between recent hospitalisations in an acute-care unit and MRSA carriage

| Table 1. Association of patients’ characteristics with MRSA carriage by univariate analysis |
|-----------------------------------------------|-------------------------------|-----------|---------|
| Characteristics                  | MRSA carriers (n = 41) | Patients (n = 109) | OR | P   |
| Male gender                       | 16/36                       | 1.54       | NS     |
| Age > 80 years                    | 25/55                       | 1.97       | NS     |
| GIR score < 3                     | 37/81                       | 4.98       | <0.01  |
| Hospitalisation in an acute-care ward within the last year | 12/25 | 1.74 | NS |
| Antimicrobial therapy within the last year | 36/66 | 8.93 | <10−3 |
| Invasive interventions within the last year | 6/16 | 0.99 | NS |
| Physical therapy within the last year | 19/52 | 0.91 | NS |
| Subcutaneous catheter             | 26/41                       | 6.01       | <0.0001|
| Feeding tube                      | 3/4                         | 5.29       | NS     |
| Urinary-tract catheter            | 7/9                         | 6.67       | <0.03  |
| Wounds, bedsores, chronic skin lesions | 12/20 | 3.07 | <0.03 |

* NS, not significant.

| Table 2. Prevalence of MRSA carriage according to antimicrobial regimens prescribed within the last 12 months |
|---------------------------------------------------------------|-------------------------------|-----------|
| Antimicrobial agents                  | Patients (prevalence) |
| Third-generation cephalosporins         | 21 (76.2%) |
| Fluoroquinolones                      | 13 (84.6%) |
| Amoxicillin + clavulanic acid           | 13 (30.8%) |
| Other β-lactams                        | 16 (43.8%) |
| Other agents                           | 19 (52.6%) |
| No antimicrobial treatment             | 44 (11.4%) |
could be explained by the potential efficacy of the program of MRSA control which has been implemented in our hospital’s acute-care wards for several years [18]. Nevertheless, the healthcare worker awareness of MRSA is probably lower in hospital settings without hospitalisation beds, like the medical imaging department. This lack of awareness could explain the strong and significant association identified in the study between medical imaging within the preceding year and MRSA carriage. To our knowledge, few data concerning the infecting risk in medical imaging are available. Some outbreaks with *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase [19] and methicillin-susceptible *S. aureus* [20] caused by ultrasonography-coupling gel contaminated have been described. The risk of gel contamination is particularly high when they are conditioned in large bottles for multiple uses. Gaillot et al. [19] have shown that *K. pneumoniae* could persist more than 4 weeks in ultrasonography-coupling gels. In a study conducted in a Nigerian teaching hospital [21], probes used for routine ultrasonography were usually contaminated with MRSA immediately after contact with patients. The absence of appropriate decontamination induces a high risk of cross-transmission to the next patient. Indeed, Bello et al. [21] have demonstrated that the average number of bacteria transmitted by the contaminated probe was significantly higher than that transmitted by the probe after decontamination. In a recent study conducted in our hospital [22], we have demonstrated that the information concerning the carrier status of 32% of MRSA patients who underwent medical imaging sessions was not transmitted by clinical wards to the personnel of the medical imaging department. Therefore, additional precautions could not be implemented for these patients, increasing the risk of cross-transmission. However, this significant association between medical imaging and MRSA carriage could also be explained by the fact that those patients may have more episodes of acute illness and therefore need more medical imaging sessions. Further studies are needed to explore the possibilities of MRSA cross-transmission by radiology or computed tomography scans. The association between subcutaneous catheters and MRSA carriage could correspond to the existence of cross-transmissions in the LTCF. Disability (GIR score < 3) and the presence of wounds or chronic skin lesions were associated with MRSA carriage by univariate analysis. A recent study [23] conducted in three German NHs showed an association between needing help in activities of daily living for up to 4 h per day and *S. aureus* carriage. In a study conducted in a NH in Turkey [24], MRSA carriage was also correlated with the presence of skin lesions.

The analysis of MRSA strains relatedness by PFGE underscores the high probability of the occurrence of recent cross-transmission into the LTCF units. In addition, some patterns were identified exclusively (4d) or predominantly (4c) in the unit B. The strains identified in each of the two major genomic groups (groups 4 and 7) are closely related, indicating the possibility of older cross-transmissions within the LTCF, with the occurrence of minor variations in the macrorestriction patterns during colonisation. Variations in PFGE macrorestriction patterns in pairs of *S. aureus* isolates, collected 4 weeks apart, from three cystic fibrosis patients attending a summer camp in Germany were also noticed by Schlichting et al. [4]. In our study, two clones representing 28 strains have disseminated in the whole LTCF. Finally, strains belonging to the 10 other genomic groups could have been acquired in other wards of our hospital or imported to our hospital and then selected from the patients’ commensal flora by antimicrobial pressure.

We identified four patients carrying two unrelated strains in different anatomical sites. A study conducted in 22 NHs in the USA demonstrated that acquisition of new persistent MRSA strains could frequently occur in MRSA carriers [25].

Several potential limitations should be considered in the interpretation of our results. First, some limitations were due to the study design. Because it was a cross-sectional study, the temporal relationship between the acquisition of MRSA and exposure to antimicrobial agents and medical imaging examination is uncertain. However, the presence of a causal relationship between previous medical imaging examination and colonisation with MRSA seems likely because of the dose-response relationship. Moreover, some carriers may have contracted their MRSA before admission to the LTCF. This constitutes a bias for interpreting results. In addition, it was impossible to determine if the transmission mainly occurred directly or indirectly.

Lastly, the influence of the environmental contamination was not studied whereas it has been identified as a potential reservoir for MRSA dissemination [26, 27].

### Table 3. Association of patients’ characteristics with MRSA carriage by multivariate analysis (final model)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard error</th>
<th>Wald chi-square</th>
<th>Adjusted odds ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.1430</td>
<td>0.3667</td>
<td>0.1520</td>
<td>0.6966</td>
<td></td>
</tr>
<tr>
<td>3-GC or fluoroquinolones</td>
<td>1.2453</td>
<td>0.3653</td>
<td>11.632</td>
<td>12.07 (5.9 – 24.7)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Other antimicrobial agent</td>
<td>0.7404</td>
<td>0.3033</td>
<td>5.9597</td>
<td>4.40 (2.43 – 7.97)</td>
<td>0.0146</td>
</tr>
<tr>
<td>Medical imaging</td>
<td>0.8131</td>
<td>0.3295</td>
<td>6.0889</td>
<td>5.08 (2.66 – 9.69)</td>
<td>0.0136</td>
</tr>
<tr>
<td>Subcutaneous catheter</td>
<td>0.5648</td>
<td>0.2561</td>
<td>4.8647</td>
<td>3.09 (1.87 – 5.10)</td>
<td>0.0274</td>
</tr>
</tbody>
</table>
recent study [28] carried out in two German intensive care units identified a significant correlation between survival times in hospital environment and the genetic diversity of various pathogens including S. aureus. The diversity identified for our MRSA strains could be related to a prolonged environmental survival. This absence of environmental investigation and the study design make uneasy the interpretation of certain results of molecular typing. For instance, it was difficult to assess why group 4 and group 7 were the two major clones isolated.

Specifically, hospital-associated risk factors of MRSA carriage like recent hospitalisation or surgical intervention were not associated with MRSA carriage in our high-prevalence LTCF. On the contrary, we identified molecular arguments of cross-transmissions inside this setting, underlining the necessity for preventive measures. We also identified arguments of MRSA selection pressure by antimicrobial treatments. Certain factors associated with carriage could represent manners of MRSA selection or cross-transmission in the community. For instance, the elderly are important medical imaging consumers outside the hospital setting. Further studies are needed to confirm and explain the strong and independent association identified between MRSA carriage and medical imaging, which could also represent an unknown mode of MRSA transmission in the community.

Key points
• Contrary to most previous data, a prevalence of MRSA carriage >30% is reported in certain LTCFs in Europe.
• In this LTCF, prior antimicrobial therapy with broad-spectrum agents is strongly associated with MRSA carriage.
• In this LTCF, MRSA transmission occurs directly or not, according to data from molecular analysis.
• Medical imaging sessions could represent a way of transmission which should be explored by further investigations.

Conflict of interest declaration
There are neither financial interest nor dual commitments that represent potential conflict of interest.

Supplementary data
Supplementary data for this article are available online at http://ageing.oxfordjournals.org.

References


Received 9 October 2007; accepted in revised form 29 November 2007