Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among residents of nursing homes in Belgium

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Objectives: A national survey was conducted to determine the prevalence, risk factors and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among nursing home (NH) residents in Belgium.

Methods: A random stratified, cross-sectional prevalence survey was conducted in NH residents who were screened for MRSA carriage by multisite enriched culture. Characteristics of NHs and residents were collected by a questionnaire survey and analysed by two-stage logistic regression modelling. MRSA isolates were genotyped by PFGE, staphylococcal cassette chromosome mec (SCCmec) typing, multilocus sequence typing (MLST) and resistance genes.

Results: Of 2953 residents screened in 60 NHs, 587 (19.9%) were MRSA carriers. Risk factors included hospital contact, antibiotic exposure, impaired mobility and skin lesions at the resident level, and lack of MRSA surveillance, lack of antibiotic therapeutic formulary and the combination of less-developed infection control activities and a high ratio of physicians to residents at the institution level. MRSA isolates showed eight major types, three of which were predominant: B2-ST45-SCCmec IV (49%; where ST stands for sequence type); A21-ST8-SCCmec IV (13%); and A20-ST8-SCCmec IV (10%). Each was recovered in 55, 21 and 25 NHs, respectively. The geographical distribution of NH genotypes paralleled that of acute-care hospitals.

Conclusions: A high prevalence of MRSA carriage in NH residents was associated with hospital care, co-morbidities and less-developed coordination of institutional care. The predominant MRSA strains from NH residents and hospitalized patients of the same area were identical. Strengthening and coordination of MRSA surveillance and control activities are warranted within and between NHs and hospitals.

Keywords: prevalence, risk factors, molecular epidemiology

Introduction

*Staphylococcus aureus* is a major human pathogen causing a wide range of diseases. During the past two decades, strains of methicillin-resistant *S. aureus* (MRSA) have increased in incidence in many parts of the world as agents of nosocomial infections. In Europe, the proportion of methicillin-resistant strains among *S. aureus* isolated from bacteraemia ranged from <3% in Scandinavian countries and in the Netherlands to >30% in southern countries and the UK between 1999 and 2002.¹ After being endemic in acute-care hospitals in the 1980s–1990s, MRSA disseminated into long-term care facilities,
constituting an increasing reservoir of carriers. In Belgium, the proportion of patients who were MRSA carriers within 48 h of hospitalization rose significantly from 15% in 1995 to 31% in 2001. Residents of nursing homes (NHs) are now considered to be at high risk of MRSA carriage. MRSA colonization in NH residents is associated with an increased mortality rate, particularly in individuals with severe impaired cognitive function.

Since the late 1990s, community-acquired (CA)-MRSA strains have been reported worldwide to cause infections in young and healthy people who lack exposure to healthcare. CA-MRSA strains are unrelated to nosocomial strains and frequently produce the Panton–Valentine leucocidin. Recently, MRSA carriage has also been reported with high frequency in people in contact with livestock animals, including farmers and veterinarians. Those MRSA strains appear unrelated to nosocomial and CA-MRSA strains.

The aims of this study were to determine the prevalence of MRSA carriage in residents of Belgian NHs, to characterize risk factors for carriage and to determine the molecular epidemiology of MRSA carriage.

**Methods**

**Study design**

A cross-sectional prevalence survey was conducted from January to September 2005. Sixty NHs (6%) with a total of 6365 beds (median 106 beds; range 38–279 beds) were selected after stratification by province (n = 11) from the national database of high-skilled NHs taking care of elderly permanent residents with important nursing care needs in Belgium. The number of eligible NHs (n = 985) taking care of elderly permanent residents with important nursing care needs was divided by 60 to obtain the sampling interval k.

A random number, x, between 1 and 16, was drawn to select the xth facility, after which every 16th facility was included. Residents were accommodated in rooms with one to four beds. On site, the study coordinator selected randomly up to 50 residents and 10 reserve residents from the residents’ registry. The total number of residents was divided by the total number of rooms to obtain the mean number of residents/room. The number of rooms to be sampled was obtained by the division of the number of residents to be selected (60 residents: 50 + 10 reserve) by the mean number of residents/room. To calculate the sampling interval, k, the total number of rooms was divided by the number of rooms that would be sampled. A random number between 1 and k was drawn to select the xth bed, after which every kth room was included. This was repeated for the NHs: region; private versus public ownership; number of beds; proportion of high-nursing-skilled beds; nurse and physician staffing level; and detailed components of antibiotic and infection control policies. The medical staffing level of NHs was measured as number of GPs per four resident beds. Missing data on the number of GPs in 13 NHs were substituted by a value predicted by the number of beds in the NHs and four other variables describing the coordination of the activities of GPs in the NHs. The infection control programme was quantified by constructing an MRSA control score by summing the value given to the following six variables: MRSA screening at admission from hospital; single room isolation of MRSA carriers; cohort nursing of MRSA carriers; and wearing of gloves, masks or apron for care of MRSA carriers. Each of these items was scored 0 if ‘never performed’ or if data were missing, 1 for ‘sometimes performed’ and 2 for ‘always performed’, resulting in a score range of 0–12. Based on the median value of this score, the MRSA control score was considered ‘low’ if <8 and ‘high’ if ≥8.

**MRSA screening**

In each NH, local nurses performed a same-day sampling of swabs from the residents’ anterior nares, throat, chronic wounds or decubitus ulcers, and urinary meatus if catheterized. Swabs were inoculated into Stuart transport medium (Copan, Italy) and sent within 24 h to the laboratory. Swabs from each resident were pooled and inoculated into brain heart infusion broth supplemented with 7.5% NaCl. After 24 h of incubation, broths were subcultured onto selective chromogenic agar (SAID; bioMérieux, France) for *S. aureus* detection. Suspect colonies of *S. aureus* were identified by the coagulase test. Oxacillin susceptibility was tested by cefoxitin disc (30 μg) (Rosco Neo-Sensitabs, Taastrup, Denmark) according to CLSI breakpoints.

MRSA were confirmed by multiplex PCR for *nuc, mecA* and 16S rDNA genes. MRSA strains were stored at −80°C in glycerol.

**Molecular typing**

Bacterial isolates were genotyped by Smal macrorestriction analysis of genomic DNA resolved by PFGE and analysed using BioNumerics software version 2.5 (Applied Maths, Belgium), as previously described. PFGE patterns were classified as previously described. PFGE profiles were compared with a national database of hospital and CA clones, as previously described.

The staphylococcal cassette chromosome mec (SCCmec) type was determined by multiplex PCR for *ccr* and *mec* complex on one representative isolate of each PFGE pattern. Based on the excellent concordance between PFGE and multilocus sequence typing (MLST), only a set of representative MRSA strains from each major epidemic PFGE type (n = 8) was analysed by MLST. Allelic profiles were determined using the MLST database (http://www.mlst.net).

**Epidemic index**

An epidemic index (E) was calculated taking into account the number of distinct MRSA genotypes for each NH with five or more MRSA carriers. This index was calculated using the formula of Simpson’s index of diversity $D$, where $E = 1 − D$.

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were genotypically related. An epidemic index of 1.0 means that all MRSA strains in an NH belonged to the same PFGE type.

**Antimicrobial susceptibility testing**

Susceptibility to antimicrobial agents was determined by using the Vitek 2 system (bioMérieux, France). Mupirocin susceptibility was tested by the agar screen method on Mueller–Hinton II medium supplemented with 4 mg/L mupirocin. The mupirocin resistance level was further determined by the Etest method (AB Biodisk, Sweden). Glycopeptide susceptibility was determined by the teicoplanin agar screen (TAS) method (5 mg/L), as recommended by the Comité de l’Antibiogramme de la Société Française de Microbiologie. Strains growing on TAS were further characterized by the Etest macromethod (AB Biodisk, Sweden): strains inhibited by both vancomycin and teicoplanin at ≥8 mg/L or by teicoplanin alone at ≥12 mg/L were considered as hetero glycopeptide-intermediate S. aureus (hetero-GISA). The genes conveying resistance to tetracycline, aminoglycosides, macrolide–lincosamide–streptogramin (MLS) and mupirocin were tested by multiplex PCR.

**Confidentiality and ethical considerations**

The study was approved by the Ethics Committee of the Scientific Institute of Public Health. Written informed consent was obtained from each resident enrolled in the study or their proxy. All data were reported anonymously with regard to patient and NH identification. Microbiological results of residents were confidentially notified to their GP.

**Statistical analysis**

Data were analysed using Stata 9.2 (Statacorp, Texas). Prevalence of MRSA carriage and its 95% confidence limits were calculated using the cluster survey analysis module. Median and interquartile ranges (IQRs) were calculated for continuous variables. Quantile regression was used to evaluate differences in MRSA prevalence by category of NH. Risk factors for colonization by MRSA in residents were first determined by univariate analysis followed by multiple logistic regression. To assess the effect of institutional variables, taking into account the patient risk profile of each institution, institutional variables were fitted in a multilevel model including all significant patient variables from the individual-level logistic regression analysis. *P* values of <0.01 were considered to be statistically significant.

**Results**

**Prevalence of MRSA colonization**

A total of 2953 residents from 60 NHs were screened between January and September 2005. The median age of the study population was 84 years (IQR, 79–90 years). The median length of stay in the NHs was 29 months (IQR, 12–60 months). Of 1500 (51%) residents who carried *S. aureus*, 587 (39%) were resistant to oxacillin. The mean-weighted prevalence of MRSA colonization in residents was 19.5% (95% CI: 16.4%–21.5%), ranging from 2.0% to 42.9% by NH.

**Risk factors for MRSA colonization**

At the level of the NH, MRSA prevalence was associated with the level of medical staffing, ranging from a mean MRSA percentage prevalence of 15.5% in NHs with <0.85 GPs per four beds to 23.9% in NHs with ≥1.2 GPs per four beds (*P* = 0.006). MRSA prevalence was also lower in institutions with a high intensity of MRSA control activities (15.7% versus 21.6% in low-intensity NHs, *P* = 0.031). The effect of the intensity of MRSA control activities, however, depended on the level of medical staffing. A statistical interaction was observed between the effect of the MRSA control index and the medical staffing level: in NHs with a high medical staffing level, a low MRSA control index was predisposing to MRSA carriage, while in those with a low staffing level, no effect was observed (*P* interaction <0.001). The MRSA prevalence by NH was not associated with the region of its location, ownership, number of beds, proportion of high-nursing-skilled beds or nurse staffing level. At the resident level, MRSA carriage was independently associated with previous hospital admission, current MRSA carriage, previous antibiotic use, impaired mobility, low autonomy status and the presence of skin lesions. In multilevel logistic regression analysis, the adjusted odds ratio for residents being an MRSA carrier was 1.57 (1.15–2.15, *P* = 0.005) when the staffing level was one or more GPs per four beds. The final multilevel model retained seven independent predictors of MRSA carriage, including the interaction between the level of medical staffing and the MRSA control index (Table 1).

**Molecular typing**

PFGE patterns of SmaI macrorestriction fragments classified 585 isolates into 24 groups and 56 types (Table 2). The SCCmec type distribution in 157 isolates representative of each PFGE pattern was as follows: type IV, *n* = 122; type II, *n* = 22; type VI, *n* = 2; type I, *n* = 2; type V, *n* = 1; and not typeable, *n* = 8. A second recombinase locus (ccrC or ccrAB4) was detected in 12 isolates of SCCmec type IV. MRSA strains belonging to clonal complexes (CCs) 45, 8 and 22 were clustered into three PFGE groups, B, A and L, respectively (Table 2). In contrast, strains from CC5 showed a high diversity of patterns, including PFGE groups C, G, I and K. Eighty-five percent of the isolates belonged to eight PFGE types, of which three were predominant: B2 (49% of total); A21 (13% of total); and A20 (10% of total). These three epidemic MRSA types were found in 55 (92%), 21 (35%) and 25 (42%) NHs, respectively.

**Geographical distribution of MRSA epidemic clones**

B2-ST45-SCCmec IV strains (where ST stands for sequence type) were widely disseminated around Belgium, but were predominantly found in the northern part of the country (Figure 1). Other strains showed a more geographically clustered distribution. A20- and A21-ST8-SCCmec IV strains were present mainly in the southern and western parts of the country. C10-ST225-SCCmec II strains were recovered only from the southern provinces. K1- and I1-ST5-SCCmec IV and G10-ST5-SCCmec II strains were found in the northern and western parts of Belgium. L1-ST22-SCCmec IV strains were isolated only in the southern part of Belgium. Comparison of the distribution of genotypes from this survey with the distribution...
of MRSA strains from a national survey of patients admitted to acute-care hospitals in 2003 showed a striking similarity in relative frequency in both settings at the province level (Figure 1).

Clonal diversity of MRSA in NHs

Fifty NHs had more than five residents colonized by MRSA. The epidemic index ranged from 0.08 to 1.00 by NH, with a median of 0.40. In nine (18%) facilities, MRSA strains had 100% identical PFGE types, suggesting active transmission inside the facilities.

Antimicrobial resistance

In addition to β-lactams, 95% of MRSA strains were resistant to ciprofloxacin, 47% to erythromycin and 26% to clindamycin. Resistance to tobramycin (35%) was more frequent than to gentamicin (<1%). Nine (1.5%) MRSA strains were classified as hetero-GISA. Only 2.7% of strains were high-level resistant to mupirocin and harboured the mupA gene. More than 90% of strains were susceptible to tetracycline (90%), fusidic acid (97%), rifampicin (99%), co-trimoxazole (99%) and linezolid (100%).

Among aminoglycoside-resistant isolates, the majority carried the ant(4') gene and a few the aac(6')-aph(2'') or aph(3') gene. Resistance to MLS was mediated by erm(A), erm(C) or both genes. Tetracycline-resistant MRSA strains harboured either the tet(M) or tet(K) gene. The antibiotic resistance phenotype and resistance gene profiles were associated with clonal type (Table 2).

Discussion

In this first national survey conducted in Belgium, we investigated the extent of the reservoir of MRSA carriers among a representative sample of elderly residents of chronic-care facilities. Risk factors were analysed and the phenotypic and genotypic features of these strains were compared with those described in acute-care hospitals and the community over the past decade.2,5 – 7,9 The high prevalence of MRSA carriage found among NH residents in our study is in contrast with the lower (<5%) prevalence of two regional surveys conducted in the Flanders region in 1997 and 2000, respectively. In other European countries, the prevalence of MRSA carriage in NHs varies markedly from 1% in Germany to >15% in Spain, France and the UK. In the USA, MRSA colonization rates of up to 40% were reported in Veterans Affairs facilities and NHs.24,25 In the present study, the MRSA prevalence varied markedly between NHs, as also observed in hospitals in Belgium and in long-term care facilities in Spain.21

The rise in the MRSA carriage rate in NH residents, from 4.9% in 1999 to 4.7% in 2000 to 19.5% in this survey, parallels the increasing prevalence of MRSA in Belgian acute-care hospitals during this period.1 Although the type of institutions in the three studies was similar, the fact that institutions in the previous studies participated on a voluntary basis while the current study included a random sample of institutions may account for part of this difference. A third factor to consider relates to the microbiological sampling and testing methods. In particular, the importance of extranasal MRSA colonization is well documented in NH residents.24 We used multisite swabbing, enrichment cultures and chromogenic agar to optimize the sensitivity of MRSA screening.

<table>
<thead>
<tr>
<th>Analysis level, risk factor</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
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<tr>
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<tr>
<td>hospital admission in previous year</td>
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<td>—</td>
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<tr>
<td>prior</td>
<td>1.33 (0.87–2.02)</td>
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<tr>
<td>current</td>
<td>3.23 (1.49–7.01)</td>
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<td>antibiotic use in previous 3 months</td>
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<td>fluoroquinolones</td>
<td>1.59 (1.17–2.17)</td>
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<tr>
<td>amoxicillin + clavulanic acid</td>
<td>1.59 (1.19–2.12)</td>
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<td>1.41 (1.14–1.74)</td>
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<tr>
<td>presence of wound or decubitus ulcer</td>
<td>1.57 (1.18–2.09)</td>
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<td>impaired autonomy reimbursement category</td>
<td>1.63 (1.19–2.23)</td>
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<td>Institutional level</td>
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<td>medical staffing level and MRSA control score</td>
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<tr>
<td>low/high (&lt;1, ≥8)</td>
<td>1.30 (0.86–1.96)</td>
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<tr>
<td>high/low (≥1, ≤8)</td>
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<td>no MRSA surveillance programme</td>
<td>1.51 (1.15–1.96)</td>
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<tr>
<td>no therapeutic formulary used</td>
<td>1.45 (1.11–1.90)</td>
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Table 1. Multilevel logistic regression analysis of risk factors of MRSA carriage in residents (n = 2953) of high-skilled NHs (n = 60), Belgium, 2005.
Table 2. Genotype, antimicrobial resistance genes and susceptibility profiles of MRSA isolates from NHs, Belgium, 2005

<table>
<thead>
<tr>
<th>PFGE typing</th>
<th>No. of isolates (%)</th>
<th>No. of NHs (%)</th>
<th>MLST ST</th>
<th>CC</th>
<th>SCCmec type</th>
<th>AME</th>
<th>tet(M)</th>
<th>tet(K)</th>
<th>erm(A)</th>
<th>erm(C)</th>
<th>Resistance profile (&gt;50% of isolates)</th>
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<tbody>
<tr>
<td>A</td>
<td>A20</td>
<td>56 (10)</td>
<td>25 (42)</td>
<td>8</td>
<td>8</td>
<td>IV</td>
<td>44</td>
<td>3</td>
<td>0</td>
<td>56</td>
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<td>A21</td>
<td>74 (13)</td>
<td>21 (35)</td>
<td>8</td>
<td>8</td>
<td>IV</td>
<td>69</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>19 CIP</td>
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<tr>
<td>B</td>
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<td>55 (92)</td>
<td>45</td>
<td>45</td>
<td>IV</td>
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<td>8</td>
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<td>13 (22)</td>
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<td>0</td>
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<td>I</td>
<td>I1</td>
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<td>IV</td>
<td>11</td>
<td>0</td>
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<td>G</td>
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AME, aminoglycoside modifying enzyme; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; TOB, tobramycin; TET, tetracycline.
Individual risk factors for MRSA colonization in NH residents include medical devices, previous antibiotic treatment, stay in hospital, skin lesions, impaired autonomy and mobility, as confirmed in the present study. Furthermore, we observed that residents appeared at lower risk in facilities with more-developed MRSA surveillance and control programmes, as well as in those using an antibiotic formulary. A high physician to resident ratio appeared to be a risk factor in centres with limited infection control activities. Although these associations should be considered with caution because of the self-reporting and incomplete nature of the data on institutional factors, they suggest that strengthening the medical coordination, antibiotic stewardship and infection control activities are warranted in these NHs.

In contrast to hospitals, only limited information is available on the molecular epidemiology of MRSA in long-term care facilities. Many studies showed the predominance of MRSA genotypes, which were also frequently observed in the neighbouring regional hospitals. In Belgium, Hoefnagels-
MRSA in Belgian nursing homes

Schuermans et al.\textsuperscript{18} found that the majority of MRSA strains belonged to the three major epidemic clones described at the same time in acute-care hospitals.\textsuperscript{7} Interestingly, our study revealed a higher clonal diversity. A diversification of MRSA strains was also observed in Belgian hospitals over this period.\textsuperscript{2} We also found that MRSA strains from NHs belonged to the same genotypes as those from patients admitted to Belgian acute-care hospitals over the past 5 years.\textsuperscript{2,9} Three major epidemic genotypes, ST45-SCC\textit{mec} IV PFGE type B2 and ST8-SCC\textit{mec} IV PFGE types A20 and A21, were predominant in both long-term and acute-care sectors.\textsuperscript{7} We did not find PFGE non-typeable strains of MRSA ST398, which have been recently described in Belgian pig farmers.\textsuperscript{8}

In the present survey, an interesting group of MLST CC5-related MRSA strains was identified, which was characterized by a high diversity of PFGE types, including two types not previously observed in Belgian acute-care hospitals. This polymorphism could reflect the ability of MRSA strains from this lineage to frequently acquire mobile genetic elements, such as SCC\textit{mec}, antibiotic resistance and toxin genes.\textsuperscript{9,10,28,29} MRSA strains of CC5 have been shown by phylogenetic studies\textsuperscript{28,29} to arise locally through horizontal transfers of the SCC\textit{mec} type into methicillin-susceptible strains that are common in local populations.\textsuperscript{10} CA-MRSA strains belonging to CC5 have been described recently in France.\textsuperscript{30} Further study should clarify whether the novel CC5 MRSA strains reported here arose within NHs or from as yet unknown sources in the community.

The MRSA strains in the present survey displayed a significant geographical diversity, with a genotype distribution by province that was similar in hospitals and NHs. This observation is consistent with local exchange of MRSA strains between care settings. High epidemic indices of local clustering of MRSA PFGE types suggests the possibility of cross-transmission within NH facilities, as reported in single-centre studies.\textsuperscript{22,27,31} Atypical strains of infrequent genotypes were found in areas bordering France, the Netherlands and Germany, where similar MRSA strains have been described.\textsuperscript{6,32,33} This finding is suggestive of the spread of MRSA strains through cross-border utilization or provision of long-term care services. As these bilateral health-care international agreements are rapidly expanding within the European Union, appropriate monitoring of cross-border spread of healthcare-associated multiresistant bacteria is needed.\textsuperscript{34}

The MRSA isolate resistance rates to aminoglycosides and MLS were lower here than in hospitals.\textsuperscript{9} We observed a low prevalence of hetero-GISA strains, as described in Belgian hospitals.\textsuperscript{9} The low rate of resistance to mupirocin reported here is similar to that found in acute-care hospitals.\textsuperscript{7} Topical mupirocin still appears useful for MRSA decolonization in these long-term care facilities.

Our point prevalence study design has inherent limitations. Firstly, it could have led to underestimation of the occurrence of intermittent carriage. Indeed, in a longitudinal study over an 8 week period, Stone \textit{et al.}\textsuperscript{25} reported that over one-third of nasal MRSA carriers were intermittent carriers. Secondly, we could not ascertain if MRSA acquisition occurred during hospitalization or stay in the long-term care facilities. MRSA-colonized residents are frequently transferred between hospitals and NHs, thereby amplifying opportunities for MRSA transmission within and between these facilities. Admission surveillance culture for early detection of MRSA carriers and implementation of barrier precautions must be coordinated between care sectors.

The incidence of MRSA infection in NHs is less well studied than MRSA colonization. While colonization with MRSA appears to be common, infection appears infrequent and was documented in only 2%–4% of residents.\textsuperscript{35} Residents colonized with MRSA had, nevertheless, a 4- to 6-fold increased risk of developing an infection compared with non-carriers,\textsuperscript{36} and experienced higher morbidity and mortality.\textsuperscript{3}

In conclusion, this first nationwide representative study demonstrates that skilled NHs host an important reservoir of MRSA carriers in Belgium. A majority of residents carry MRSA strains identical to those from hospitals in the same region. MRSA strains of CC5 showed a high degree of PFGE polymorphism as well as horizontally acquired resistance or virulence genes, consistent with a divergent evolution in populations under different selective pressures.\textsuperscript{26,29} A better understanding of the dynamics of the MRSA reservoir in NHs and the direction of flux of colonized patients between acute- and chronic-care sectors should inform future control policies.

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Transparency declarations

None to declare.

References


\textsuperscript{3} Suetens C, Niclaes L, Jans B \textit{et al.} Methicillin-resistant \textit{Staphylococcus aureus} colonization is associated with higher mortality.


