Epidemiol. Infect. (2013), **141**, 1199–1206. © Cambridge University Press 2012
doi:10.1017/S0950268812001999

Prevalence, risk factors and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in residents of long-term care facilities in Luxembourg, 2010

J. MOSSONG[1]*, E. GELHAUSEN[2], F. DECRUYENAERE[3], A. DEVAUX[1], M. PERRIN[3], J. EVEN[4] AND E. HEISBOURG[2]

[1] *Surveillance & Epidemiology of Infectious Diseases, National Health Laboratory, Luxembourg*
[2] *Curative Medicine, Health Directorate, Luxembourg*
[3] *Bacteriology, National Health Laboratory, Luxembourg*
[4] *Serology & Virology, National Health Laboratory, Luxembourg*

Received 21 May 2012; Final revision 9 August 2012; Accepted 9 August 2012; first published online 7 September 2012

SUMMARY

A prevalence survey of methicillin-resistant *Staphylococcus aureus* (MRSA) was performed in 2010 in 19 long-term care facilities in Luxembourg. Of the 954 participating residents, 69 (7·2%) were colonized by MRSA. Previous history of MRSA [odds ratio (OR) 7·20, 95% confidence interval (CI) 3·19–16·27], quinolone therapy in the previous year (OR 2·27, 95% CI 1·17–4·41) and $\geqslant$24 h care administered per week (OR 4·29, 95% CI 1·18–15·56) were independent risk factors for MRSA colonization. More than 75% of strains were of clonal complex (CC)5, mainly *spa*-type t003 or sequence type (ST)225 and ST710, which is a rapidly emerging lineage prevalent in central Europe. Five residents were colonized by livestock-associated genotypes belonging to CC398. Previously dominant CC8 strains have recently been replaced by more resistant CC5 strains in Luxembourg.

Key words: Antibiotic resistance, antimicrobial resistance in agricultural settings, hospital-acquired (nosocomial) infections, methicillin-resistant *S. aureus* (MRSA), molecular epidemiology.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for a substantial burden of illness due to healthcare- and community-associated infections. Within the European Union it is estimated that 150 000 patients per year have clinically relevant MRSA infections resulting in extra annual costs of €380 million [1]. Over the past two decades, there has been a shift away from acute-care hospitals with more healthcare being delivered in the outpatient setting, either at home or in long-term care facilities (LTCFs) [2, 3]. Studies from different European countries have shown variable prevalence rates of MRSA colonization and predominance of MRSA genotypes often reflecting the national or local MRSA prevalence in acute-care hospitals [4–16].

The aim of our study was to investigate the role of LTCFs as potential reservoirs for MRSA in the community. We conducted a prevalence study of MRSA colonization in a nationally representative sample of elderly residents of LTCFs in Luxembourg, to determine risk factors for colonization and to

* Author for correspondence: Dr J. Mossong, Surveillance & Epidemiology of Infectious Diseases, National Health Laboratory, 42 rue du Laboratoire, L-1911 Luxembourg, Luxembourg. (Email: joelmossong@gmail.com)

conduct molecular epidemiology of MRSA colonization isolates in the long-term care setting.

METHODS

Study population and epidemiological data collection

A nationally representative cross-sectional MRSA prevalence study in LTCF facilities was conducted from February to June 2010 in the Grand Duchy of Luxembourg. At the beginning of the study, in January 2010, 48 LTCFs with a total of 4688 beds (mean 98 beds per LTCF) were accredited by the Ministry of Family Affairs. According to national statistics (http://www.statistiques.public.lu), Luxembourg's elderly population (aged $\geqslant$65 years) consisted of 70 046 persons in 2010, so that 67 beds/1000 elderly persons were available in publicly registered institutions.

Twenty-six LTCFs were selected at random using the random number generation function in Excel (Microsoft Corporation, USA) on three sequential sampling occasions until an approximate target of 1000 participants were recruited. Following an invitation by the Health Directorate, 19 (73%) LTCFs agreedto participate in the study. While one of the 19 LTCFs was physically adjacent to and associated with a hospital, the other LTFCs were not, and were geographically dispersed in different regions.

All residents of the nursing homes were informed by their local staff about the study and invited to participate subject to written informed consent given either by the elderly residents themselves or by an appropriate family member. The prevalence rate within the institution was communicated to individual LTCFs, but not individual test results. At the time of the study, there were no national guidelines on control procedures for positive MRSA residents. Ethical approval for the study was obtained from the national ethics committee. Demographic and risk factor data from all residents were collected using an anonymous paper questionnaire which was completed by nurses in the LTCFs prior to swab sampling. Data included date of sampling, date of residence, age in categories, sex, history of previous MRSA and decontamination, history of antibiotic therapies within the past year, hospitalization, comorbidities [chronic wounds, pressure sores (decubitus), urinary incontinence, urethral catheter, suprapubic catheter, diabetes, disorientated/dementia] and time allocated to essential care (minutes per week) according to the formal care assessment by the national dependency insurance.

Microbiological methods

For each resident, separate swabs for nose and throat were taken by the same nurse of the Health Directorate (in 18 LTCFs, respectively, in one LTCF by the infection control nurse) and additional swabs were taken if wounds were present. Urine samples were taken if the resident had a urethral or suprapubic catheter. Samples were transported on the same day to the laboratory for testing. Following incubation of pooled swabs in Todd-Hewitt enrichment broth (bioMérieux, France) for 18–24 h at 35 °C, 10 μl broth was plated onto chromID MRSA agar (bioMérieux) and incubated for 18–24 h at 35 °C. Putative green MRSA isolates were biochemically confirmed with the ID32 Staph gallery (bioMérieux). Following genomic DNA extraction by NucliSENS easyMAG (bioMérieux) an in-house multiplex real-time PCR on the LightCycler II platform (Roche Diagnostics, Germany) was used to simultaneously detect the presence of *nuc* and *mecA* genes, Panton–Valentine leukocidin (PVL) toxin coding gene and Toxic Shock Syndrome Toxin 1 (TSST-1) coding gene (see the Supplementary Material for a more detailed description). Isolates were tested by the disk diffusion method and the SIRSCAN automatic reader (i2a, Perols, France) for susceptibility to penicillin, cefoxitin, kanamycin, tobramycin, gentamicin, erythromycin, clindamycin, ciprofloxacin, rifampicin, tetracycline, fosfomycin, vancomycin, cotrimoxazole, furanes and mupirocin using CLSI breakpoints.

Molecular typing

All isolates were *spa*-typed using PCR conditions and the spa-F2 forward primer as described previously [17–19]. Analysis of *spa* sequences and assignment of *spa*-types were performed using the *spa*-typing plug-in tool of BioNumerics 5.10 (Applied Maths, Belgium). In addition, a selection of strains (one isolate of a particular *spa*-type per LTCF) was typed by multilocus sequence typing (MLST) [20].

Statistical analysis

All statistical testing was performed using Stata 10.1 (StataCorp., USA). Variation of the prevalence of MRSA colonization by LTCFs was assessed using StatXact-4 (Cytel Software Corp., USA). Risk factors for MRSA colonization were first estimated by univariate analysis and then by multiple logistic regression.

Table 1. *Number of residents invited to participate, participants and MRSA-positive residents by long-term care facility (LTCF)*

LTCF	Residents	Participants	MRSA-positive	Participation rate (%)	Prevalence rate (%)
A	70	43	3	61·4	7·0
B	56	24	2	42·9	8·3
C	79	12	2	15·2	16·7
D	154	71	8	46·1	11·3
E	200	144	17	72·0	11·8
F	100	35	1	35·0	2·9
G	100	69	2	69·0	2·9
H	108	44	1	40·7	2·3
I	59	24	3	40·7	12·5
J	144	130	12	90·3	9·2
K	143	63	4	44·1	6·3
L	132	72	5	54·5	6·9
M	43	20	1	46·5	5·0
N	80	23	3	28·8	13·0
O	126	42	0	33·3	0·0
P	85	16	1	18·8	6·3
Q	100	50	3	50·0	6·0
R	111	42	0	37·8	0·0
S	72	30	1	41·7	3·3
Total	**1962**	**954**	**69**	**48·6**	**7·2**

RESULTS

In 19 LTCFs, 1962 residents (mean 103 per LTCF) were invited to participate. Swab samples with epiemiological questionnaires were collected from 954 residents yielding an overall effective participation rate of 48·6%. Participation varied significantly by LTCF (see Table 1) with a range from 15·2% to 90·3% (Pearson's χ^2, $P<0·0001$). Women comprised 71·3% of the participants and 46% were aged $\geqslant$85 years. Overall, 69 residents were colonized by MRSA yielding a prevalence rate of 7·2% [95% confidence interval (CI) 5·7–9·1]. MRSA colonization did not vary significantly between LTCFs (range 0–16·7%; Monte Carlo estimate of exact P value of Pearson's χ^2 test, $P=0·185$).

In univariate analysis, significant risk factors for MRSA colonization were length of stay [odds ratio (OR) of starting residency before 2007: 1·93, 95% CI 1·16–3·20], known history of MRSA (OR 9·30, 95% CI 4·49–19·29), weekly care administered ($\geqslant$24 h: OR 8·08, 95% CI 3·26–20·00), any antibiotic therapy in the preceding year (OR 3·87, 95% CI 2·20–6·82), β-lactam therapy in the preceding year (OR 2·34, 95% CI 1·39–3·96), quinolone therapy in the preceding year (OR 2·74, 95% CI 1·63–4·61), pressure sores (OR 4·01, 95% CI 1·75–9·20), urinary incontinence (OR 2·30, 95% CI 1·39–3·81) and suprapubic catheter (OR 3·96, 95% 1·73–9·07). Age, sex, hospitalization in the preceding year, macrolide therapy in the preceding year, chronic wounds, urethral catheter, diabetes and disorientation were not associated with MRSA colonization ($P>0·05$) in univariate analysis.

In multivariate analysis (Table 2), previous history of MRSA (OR 7·20, 95% CI 3·19–16·27), quinolone therapy in the previous year (OR 2·27, 95% CI 1·17–4·41) and $\geqslant$24 h care administered per week (OR 4·29, 95% CI 1·18–15·56) remained independently associated with MRSA colonization.

For the 69 isolates submitted to *spa*-typing, 17 different *spa*-types were obtained (Supplementary Table S2). One isolate was not typable because the sequence of one of its repeats was too short for type assignment. Thirty-nine (57·3%) isolates belonged to *spa*-type 003, the remaining *spa*-types all accounting for $<$5% of isolates. The distribution of *spa*-types differed significantly by LTCF (exact P value = 0·0308), indicating a certain clustering effect and possibly transmission within LTCFs.

MLST on 31 isolates yielded eight different sequence types belonging to six clonal complexes (CC): CC5 (21 isolates), CC8 (four isolates), CC22 (two isolates), CC45 (one isolate), CC59 (one isolate) and CC398 (two isolates). Interestingly isolates of

https://doi.org/10.1017/S0950268812001999 Published online by Cambridge University Press

Table 2. *Univariate and multivariate logistic regression analysis of risk factors associated with MRSA colonization in long-term care facility residents*

	Proportion of residents (%) with MRSA colonization	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
History of MRSA					
Yes	17/43 (39·5%)	9·30 (4·49–19·29)	<0·001	7·20 (3·19–16·27)	<0·001
No	26/396 (6·6%)	1	–	1	–
Unknown	25/451 (5·5%)	0·84 (0·47–1·47)	0·533	1·04 (0·60–1·83)	0·875
Missing data	1/64 (1·6%)	0·23 (0·03–1·69)	0·148	0·19 (0·04–0·97)	0·046
Hours of care per week					
None	7/274 (2·5%)	1	–	1	–
<12 h	17/267 (6·4%)	2·59 (1·06–6·36)	0·037	2·20 (0·74–6·58)	0·158
12–24 h	24/248 (9·7%)	4·09 (1·73–9·66)	0·001	2·46 (0·83–7·26)	0·105
>24 h	18/103 (17·5%)	8·08 (3·26–20·00)	<0·001	4·29 (1·18–15·56)	0·027
Missing data	3/62 (4·8%)	1·94 (0·49–7·72)	0·347	1·91 (0·54–6·79)	0·318
β-lactams in past year					
Yes	24/187 (12·8%)	2·37 (1·40–4·00)	0·001	1·56 (0·82–2·99)	0·177
No	44/751 (5·9%)	1	–	1	–
Missing data	1/16 (6·3%)	1·07 (0·14–8·30)	0·947	2·01 (0·18–22·37)	0·571
Quinolones in the past year					
Yes	25/177 (14·1%)	2·75 (1·63–4·63)	<0·001	2·27 (1·17–4·41)	0·015
No	43/761 (5·6%)	1	–	1	–
Missing data	1/16 (6·3%)	1·11 (0·14–8·63)	0·918	–	–
Pressure sores					
Yes	8/36 (22·2%)	4·01 (1·75–9·20)	0·001	1·61 (0·47–5·51)	0·444
No	58/873 (6·6%)	1	–	1	–
Missing data	3/45 (6·7%)	1·00 (0·30–3·34)	0·995	0·78 (0·28–2·18)	0·640
Urinary incontinence					
Yes	39/363 (10·7%)	2·30 (1·39–3·81)	0·001	1·28 (0·51–3·19)	0·598
No	28/563 (5·0%)	1	–	1	–
Missing data	2/28 (7·1%)	1·47 (0·33–6·51)	0·612	1·33 (0·07–25·55)	0·851
Suprapubic catheter					
Yes	8/36 (22·2%)	3·96 (1·73–9·07)	0·001	1·30 (0·59–2·87)	0·517
No	60/892 (6·7%)	1	–	1	–
Missing data	1/26 (3·9%)	0·55 (0·07–4·16)	0·567	0·97 (0·04–21·04)	0·982
Total	69/954 (7·2%)				

OR, Odds ratio; CI, confidence interval.

spa-type t003 could be further discriminated by MLST into ST710 (four isolates) and ST225 (14 isolates). Five (7·2%) isolates belonged to livestock associated *spa*-types t899 and t3106: one isolate of each of these two *spa*-types were confirmed as having sequence type (ST)398.

All isolates were resistant to β-lactams, 96% to ciprofloxacin, 75% to kanamycin and tobramycin, 72% to erythromycin, 68% to clindamycin, 10% to mupirocin, 7% to cotrimoxazole and tetracycline. None of the isolates was resistant to gentamicin, rifampicin and furanes. Resistance to the kanamycin, tobramycin, erythromycin and clindamycin was associated with CC5 (exact χ^2 test, $P<0·001$) and resistance to tetracycline and cotrimoxazole with CC398 (exact χ^2 test, $P<0·001$). Six of seven isolates resistant to mupirocin were clustered in a single institution.

DISCUSSION

The MRSA colonization prevalence of 7·2% observed in the current study in Luxembourg ranks in the middle of prevalences reported by similar studies in other European countries since 2000 (Table 3); our prevalence rate was generally lower than in Belgium

https://doi.org/10.1017/S0950268812001999 Published online by Cambridge University Press

Table 3. *Compilation of MRSA prevalence studies in long-term care facility/nursing home residents in Europe published on PubMed between 2000 and 2011*

Country – region	Year	Prevalence	Number of LTCFS	Predominant genotypes	Independent risk factors	Ref.
Germany – North East	2003	0%	3	ND	Not comparable (no multivariate analysis done)	[4]
Germany – South	1999–2000	1·1%	47	CC5	Wounds, urinary catheters, limited mobility, hospitalization, medium size LTCF	[5]
Germany – Central	2008	2·3%*	5	CC5–CC22	Haemodialysis, recent acute infection before hospital admission*	[6]
Germany – West	2000–2001	3%	61	CC45		[7]
Belgium – North	2000	4·7%	24	ND	Hospitalization, fluoroquinolone and nitrofurane, >2 beds per room, patient mobility, urinary catheter, pressure sure, short length of stay, underlying disease	[8]
Luxembourg	**2010**	**7·2%**	**19**	**CC5**	**History of MRSA, quinolone therapy, ⩾24 h of care per week**	**This study**
Germany – North	2009	7·6%	32	CC22	Urinary tract catheters, wounds, previous hospital admission, and high grade resident	[9]
Italy – North	2006	7·8%	2	NC	Cancer, hospitalization, antibiotics	[10]
UK – West England	2008–2009	7·8%	51	ND	Not comparable (different control groups)	[11]
Slovenia	2001	9·3%	1	ND	Antibiotics, hospitalization	[12]
Spain – Catalonia, Balearic islands	2005	15·5%	9	ND	Age ⩾85 years, comorbidities, pressure sores, antibiotics, medical devices, small size LTCF, transfer from hospital	[13]
Belgium	2005	19·9%	60	CC45, CC8	Hospitalization, current MRSA carriage, fluoroquinolones, amoxicillin + clavulanic acid, impaired mobility, wound or decubitus ulcer, dependency status	[14]
UK – North England	2005	22%	39	ND	Low ratio of nurses to beds, deprived area, male gender, invasive device, hospitalization	[15]
France	2004	37·6%	1†	NC	Fluoroquinolones, other antibiotics, medical imaging, subcutaneous catheter	[16]

LTCF, Long-term care facility.

* This study was conducted in hospitals, nursing homes and geriatric rehabilitation centres. The prevalence indicated refers to nursing homes only, but risk factors indicated in multivariate analysis are not limited to nursing home residents.

† Study was conducted in a geriatric unit associated with a teaching hospital.

and France, but generally higher than in Germany. Direct comparisons between countries and studies are difficult, because of differing sampling and microbiological methodologies, differing 'case-mix' populations, and possibly also because of different financing mechanisms of dependency care and LTCF provision. Due to their small number and limited geographical scope, studies presented in Table 3 are unlikely to yield a representative picture of the actual MRSA colonization rates in LTCFs throughout Europe. Difficulties in comparing MRSA prevalence rates in hospitals and other healthcare institutions included differences in timing of screening (on admission or during hospital stay), selection criteria (all admissions or patients at high risk for MRSA) and anatomical sampling sites [21].

LTCFs in Luxembourg are classified into two types: 'maison de soins' (or nursing homes using the terminology of Ribbe *et al*. [22]) which offer a higher level of care to mainly dependent and frail elderly with chronic diseases, disabilities and/or dementia, and CIPA ('centre intégré pour personnes âgées' or integrated centre for the elderly) whose residents at admission more often require no or very little care, but who may remain in the same institution if the care-load increases as they get older. CIPAs are thus a mixture of the two categories of nursing and residential homes according to the terminology of Ribbe *et al*. [22]. Given that a large proportion of residents in our study came from such CIPAs (LTCFs were selected at random in our study), it is clear that the average dependency level was probably lower than in the Belgian study which focused on nursing homes only [14]. The MRSA colonization rate in highly dependent residents (needing $\geqslant$24 h of care per week, Table 2) in our study was 17·5% which is closer to the prevalence reported in Belgium.

In a recent national MRSA prevalence survey conducted in Luxembourgish hospitals in 2008, a lower prevalence rate of 3·7% was found (A. M. Ternes & J. C. Schmit, unpublished report) suggesting that asymptomatic carriage in LTCFs is likely to be an important reservoir for MRSA. Again comparisons between prevalence rates and hospitals should be interpreted with caution, as screening in hospitals was performed on admission, whereas our study participants were sampled on average a long time after admission.

While four out of five major hospital-acquired MRSA clonal complexes (CC5, CC8, CC22, CC30, CC45) [23, 24] were detected in our study, we observed a large predominance (>75%) of strains belonging to CC5 (mainly of *spa*-type t003 or variants thereof, corresponding to ST225, ST710 or more rarely ST5). This dominance of CC5 strains was also found in the national hospital survey in Luxembourg in 2008, although to a lesser extent: t003 or variants thereof corresponded to 65% of isolates. This is in marked contrast to the MRSA genotype distribution in Luxembourg in 2003, when strains belonging to CC8 (*spa*-type t008) were largely predominant in hospitals [25]. Thus we have observed at a rather rapid time-scale a local strain replacement by a new lineage that has recently emerged in hospitals in central Europe (Germany, Denmark, Switzerland, Czech Republic) [26]. Why this rapid strain emergence has taken place is unclear. However, it is interesting to note that in comparison to previously prevalent CC8 strains, currently dominating CC5 strains tend additionally to be resistant to clindamycin, erythromycin and tobramycin. In our study, reported treatment with these antibiotics was rare and colonization with MRSA was only associated with fluoroquinolones and almost all strains belonging to CC5 and CC8 shared this phenotype. This illustrates that the epidemiology of MRSA in Europe and elsewhere is constantly changing and that molecular surveillance shared via the internet is crucial to provide early warning of emerging strains, cross-border spread and importation by travel [27].

Our study had several limitations. Despite a relatively high consent rate (49%), the use of informed consent most likely led to a selection bias in the patients sampled which could have led to an underestimate of the prevalence of MRSA. The wide range of the participation rate between LTCFs was probably to some extent due to the relative priority that our study was given by the various LTCF management staff who were instructed to inform residents by providing them with study leaflets and explaining the purpose of the study. Moreover, because of the cross-sectional nature of the study, transmission within LTCFs could not be assessed. Clustering of *spa*-types by LTCF, for example, could be also due to referral biases from different hospitals or geographical areas which have distinct *spa*-types.

To the best of our knowledge our study is the first to report recently identified livestock associated-MRSA belonging to CC398 [28] in LTCF residents. Whether residents were colonized prior to entering or during residency cannot be easily determined because we did not collect any information on contact with

livestock, farms or handling raw meat [29]. It should, however, be noted that the LTCFs where these strains were detected were located in more rural areas.

While we saw some evidence of MRSA transmission occurring within institutes, the typing methodology used in our study (*spa*-typing, MLST and antibiotic resistance typing) was not sufficiently discriminant to investigate chains of transmission, particularly for the highly prevalent t003 strains. To address this, we plan to conduct full genome sequencing [30] in order to develop single nucleotide polymorphism assays that can differentiate prevalent clones of CC5 strains further. However, we did observe a small degree of clustering, particularly of mupirocin resistance in one LTCF, but it is unclear whether this was due to transmission of resistant strains or multiply acquired resistance due to antibiotic selection pressure.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268812001999.

ACKNOWLEDGEMENTS

We thank the participating residents, staff at nursing homes and members of the national group for the prevention of nosocomial infections (GNPIN) for comments and support. This study was financially supported by a research grant from the Luxembourg government.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Köck R, *et al.*** Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Eurosurveillance* 2010; **15**: 19688.
2. **Manzur A, Gudiol F.** Methicillin-resistant *Staphylococcus aureus* in long-term-care facilities. *Clinical Microbiology and Infection* 2009; **15** (Suppl. 7): 26–30.
3. **Manzur A, *et al.*** Clinical significance of methicillin-resistant *Staphylococcus aureus* colonization in residents in community long-term-care facilities in Spain. *Epidemiology and Infection* 2012; **140**: 400–406.
4. **Daeschlein G, *et al.*** Risk factors for *Staphylococcus aureus* nasal carriage in residents of three nursing homes in Germany. *Journal of Hospital Infection* 2006; **63**: 216–220.
5. **von Baum H, *et al.*** Risk factors for methicillin-resistant *Staphylococcus aureus* carriage in residents of German nursing homes. *Infection Control and Hospital Epidemiology* 2002; **23**: 511–515.
6. **Woltering R, *et al.*** Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients in long-term care in hospitals, rehabilitation centers and nursing homes of a rural district in Germany. *Deutsche Medizinische Wochenschrift* 2008; **133**: 999–1003.
7. **Neuhaus B, *et al.*** Survey of methicillin-resistant *Staphylococcus aureus* in long-term facilities for the aged in Northrhine Westphalia. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* 2002; **45**: 898–904.
8. **Suetens C, *et al.*** Determinants of methicillin-resistant *Staphylococcus aureus* carriage in nursing homes. *Age and Ageing* 2007; **36**: 327–330.
9. **Pfingsten-Würzburg S, *et al.*** Prevalence and molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in nursing home residents in northern Germany. *Journal of Hospital Infection* 2011; **78**: 108–112.
10. **Brugnaro P, *et al.*** Clustering and risk factors of methicillin-resistant *Staphylococcus aureus* carriage in two Italian long-term care facilities. *Infection* 2009; **37**: 216–221.
11. **Lasseter G, *et al.*** *Staphylococcus aureus* carriage in care homes: identification of risk factors, including the role of dementia. *Epidemiology and Infection* 2010; **138**: 686–696.
12. **Vovko P, *et al.*** Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* in a long-term-care facility in Slovenia. *Infection Control and Hospital Epidemiology* 2005; **26**: 191–195.
13. **Manzur A, *et al.*** Prevalence of methicillin-resistant *Staphylococcus aureus* and factors associated with colonization among residents in community long-term-care facilities in Spain. *Clinical Microbiology and Infection* 2008; **14**: 867–872.
14. **Denis O, *et al.*** Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among residents of nursing homes in Belgium. *Journal of Antimicrobial Chemotherapy* 2009; **64**: 1299–1306.
15. **Barr B, *et al.*** Prevalence of methicillin-resistant *Staphylococcus aureus* colonization among older residents of care homes in the United Kingdom. *Infection Control and Hospital Epidemiology* 2007; **28**: 853–859.
16. **Eveillard M, *et al.*** Methicillin-resistant *Staphylococcus aureus* carriage in a long-term care facility: hypothesis about selection and transmission. *Age and Ageing* 2008; **37**: 294–299.
17. **Oliveira DC, *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*. *Journal of Clinical Microbiology* 2001; **39**: 574–580.
18. **Koreen L, *et al.*** spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for

use of a single marker to detect genetic micro- and macrovariation. *Journal of Clinical Microbiology* 2004; **42**: 792–799.

19. **Baum C, *et al.*** Non-spa-typeable clinical *Staphylococcus aureus* strains are naturally occurring protein A mutants. *Journal of Clinical Microbiology* 2009; **47**: 3624–3629.
20. **Enright MC, *et al.*** Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of Clinical Microbiology* 2000; **38**: 1008–1015.
21. **Dulon M, *et al.*** MRSA prevalence in European healthcare settings: a review. *BMC Infectious Diseases* 2011; **11**: 138.
22. **Ribbe MW, *et al.*** Nursing homes in 10 nations: a comparison between countries and settings. *Age and Ageing* 1997; **26** (Suppl. 2): 3–12.
23. **Deurenberg RH, Vink C, Kalenic S, *et al.*** The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection* 2007; **13**: 222–235.
24. **Wolters M, *et al.*** MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages. *International Journal of Medical Microbiology* 2011; **301**: 64–68.
25. **Schuh D.** Using spa-typing to study MRSA isolated in the Grand-Duchy of Luxembourg [in French]. Liège, Belgium: Haute école Mosane d'enseignment supérieur, 2006.
26. **Nubel U, *et al.*** A timescale for evolution, population expansion, and spatial spread of an emerging clone of methicillin-resistant *Staphylococcus aureus*. *PLoS Pathogens* 2010; **6**: e1000855.
27. **Grundmann H, *et al.*** Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Medicine* 2010; **7**: e1000215.
28. **van Loo I, *et al.*** Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerging Infectious Diseases* 2007; **13**: 1834–1839.
29. **Waters AE, *et al.*** Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clinical Infectious Diseases* 2011; **52**: 1227–1230.
30. **Harris SR, *et al.*** Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 2010; **327**: 469–474.