Postprint Version	1.0
Journal website	http://linkinghub.elsevier.com/retrieve/pii/S1473-3099(13)70036-7
Pubmed link	http://www.ncbi.nlm.nih.gov/pubmed/23473661
DOI	10.1016/S1473-3099(13)70036-7

Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *S aureus*, in nine European countries: a cross-sectional study

CASPER DJ DEN HEIJER, MD[A], EVELIEN ME VAN BIJNEN, MSC[B], W JOHN PAGET, PHD[B, C], PROF MIKE PRINGLE, MD[D], PROF HERMAN GOOSSENS, MD[E], PROF CATHRIEN A BRUGGEMAN, PHD[A], PROF FRANÇOIS G SCHELLEVIS, MD[B, F], DR ELLEN E STOBBERINGH, PHD[A,], AND THE APRES STUDY TEAM

[a] Department of Medical Microbiology, Maastricht University Medical Centre/CAPHRI, Maastricht, Netherlands
[b] NIVEL, The Netherlands Institute for Health Services Research, Utrecht, Netherlands
[c] Department of Primary and Community Care, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
[d] Division of Primary Care, University of Nottingham, Nottingham, UK
[e] Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium
[f] Department of General Practice and Elderly Care Medicine/EMGO+ Institute, VU University Medical Centre, Amsterdam, Netherlands

SUMMARY

Background: Information about the prevalence of *Staphylococcus aureus* resistance to antimicrobial drugs has mainly been obtained from invasive strains, although the commensal microbiota is thought to be an important reservoir of resistance. We aimed to compare the prevalence of nasal *S aureus* carriage and antibiotic resistance, including meticillin-resistant *S aureus* (MRSA), in healthy patients across nine European countries.

Methods: In this cross-sectional study, nasal swabs were obtained from 32 206 patients recruited by family doctors participating in existing nationwide family doctor networks in Austria, Belgium, Croatia, France, Hungary, Spain, Sweden, the Netherlands, and the UK. Eligible patients were aged 4 years or older (≥18 years in the UK) and presented with a non-infectious disorder. Swabs were sent to national microbiological laboratories for identification and isolation of *S aureus*. Antibiotic resistance testing was done at one central microbiological laboratory. We established the genotypic structure of the isolated MRSA strains with the *spa* typing method.

Findings: *S aureus* was isolated from 6956 (21·6%) of 32 206 patients swabbed. The adjusted *S aureus* prevalence for patients older than 18 years ranged from

12·1% (Hungary) to 29·4% (Sweden). Except for penicillin, the highest recorded resistance rate was to azithromycin (from 1·6% in Sweden to 16·9% in France). In total, 91 MRSA strains were isolated, and the highest MRSA prevalence was reported in Belgium (2·1%). 53 different *spa* types were detected—the most prevalent were t002 (n=9) and t008 (n=8).

Interpretation: The prevalence of *S aureus* nasal carriage differed across the nine European countries assessed, even after correction for age, sex, and family doctor. Generally, the prevalence of resistance, including that of MRSA, was low. The MRSA strains recorded showed genotypic heterogeneity, both within and between countries.

Funding: European Commission, 7th Framework Programme(grant agreement 223083).

INTRODUCTION

Staphylococcus aureus forms part of the human microbiota, and the most consistent body site from which it can be isolated is the anterior nares. [1 and 2] In addition to the commensal role of this bacterium, *S aureus* can cause various infections in human beings, ranging from minor skin infections to severe pneumonia. [3]

The potential of *S aureus* to acquire resistance has been exemplified by the rapid emergence of resistance to penicillin and meticillin. The high rates of meticillin-resistant *S aureus* (MRSA) reported during the past few decades have been especially concerning because of the paucity of alternative treatment options. MRSA is the most important cause of antibiotic-resistant health-care-associated infections worldwide. [4] Antibiotic use is believed to be the main driver for antibiotic resistance. [5]

Studies of the prevalence of *S aureus* resistance have so far mainly focused on invasive isolates, often obtained from patients admitted to hospital with bloodstream infections. [6] Because of the scarcity of resistance data from the outpatient setting, the prevalence of resistance in invasive strains has been translated into guidelines for empirical antibiotic treatment of community-acquired infections, which will promote excessive use of broad-spectrum antibiotics in outpatients. [6] An individual's commensal microbiota is thought to be the reservoir for antibiotic resistance genes and, therefore, resistance in this microbiota usually precedes the acquisition of resistance in pathogens. [7] For these reasons, the amount of resistance in the commensal microbiota should be taken into account in clinical guidelines for general practitioners for the control of antibiotic resistance at the source. [8]

In our study (Appropriateness of Prescribing Antibiotics in Primary Health Care in Europe with Respect to Antibiotic Resistance; APRES), one of the main objectives was to establish the prevalence of antibiotic resistance to commensal *S aureus*, including rates of MRSA, in nine European countries with different rates and patterns of antibiotic prescription behaviour, [9] to get an indication of the selection pressure that antibiotics exert on the general population. [8] We also aimed to assess whether differences exist in the prevalence of nasal *S aureus* carriage in the nine participating countries. Additionally, the population structure of the isolated MRSA strains was to be established with *spa* typing to detect possible clustering of specific MRSA strains within one or more countries.

METHODS

Study design

The APRES study design has been described elsewhere.[9] Therefore, we will only briefly mention the methods used. The participating family doctors (with an aim for 20 per country) were recruited from existing national networks of family doctors in Austria, Belgium, Croatia, France, Hungary, Spain, Sweden, the Netherlands, and the UK from November, 2010, to August, 2011. Every family doctor was asked to collect nasal swabs from 200 patients, aged 4 years or older (or ≥18 years in the UK because of ethics committee constraints), who visited their practice for a non-infectious disorder. Patients who had been prescribed antimicrobial drugs or who had been admitted to hospital in the previous 3 months were excluded, as were immunocompromised patients (eg, those with diabetes mellitus) and nursing home residents. The exclusion criteria were intended to guarantee the participation of generally healthy patients as a proxy for the general population. In addition to the collection of a nasal swab, every participant's background information (ie, age and sex) was recorded.

A recruitment update was done every 2 weeks to ensure equal stratification with respect to sex and age groups—ie, children (4–19 years), adults (aged 20–64 years), and elderly patients (≥65 years of age).

Ethics approval for the study was obtained in all participating countries and all patients provided written informed consent before inclusion.

Procedures

Before the start of the study, participating family doctors received a protocol for the sampling of the nasal swabs, which was explained to them further by the national family doctor coordinator or study nurse. Both anterior nares of the patients were swabbed with a charcoal swab (Transystem, 114 C; Copan Italia, Brescia, Italy). The swabs were sent to one national microbiological laboratory for isolation and identification of *S aureus*. All laboratories in the APRES study used a standardised protocol for the identification of *S aureus*. [9] Before the start of the study, the quality of the *S aureus* identification procedures of every laboratory had been assessed by sending of a masked panel of swabs, and the identification results were reported back to the microbiological laboratory of Maastricht University Medical Centre, Netherlands.

When the analysis of the collected swabs had been completed by the national laboratory, the isolated *S aureus* strains were frozen and sent in batches to the microbiological laboratory of Maastricht University Medical Centre for antibiotic resistance testing. The resistance of *S aureus* strains was established with the microdilution method for azithromycin, ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, oxacillin, penicillin, tetracycline, co-trimoxazole, and vancomycin. The control strain was *S aureus* ATCC 29213. Methods were in accordance with EUCAST guidelines, and EUCAST epidemiological cutoffs were used as resistance breakpoints. [10]

All isolates susceptible to clindamycin and resistant to erythromycin were tested for inducible clindamycin resistance with the *D* test. [11] In the case of a positive *D* test, strains were judged to be resistant to clindamycin.

All isolates with minimum inhibitory concentration (MIC) values of 1 mg/L or higher to oxacillin were analysed for the presence of the *mecA* gene with real-time PCR. [12] Real-time PCR for the *S aureus*-specific genes *femA* and *SA442* provided the internal control. [3]
Real-time amplification of the *spa* locus, with subsequent sequencing, was done as described previously. [13] The *spa* types were clustered into *spa* clonal complexes with the Based Upon Repeat Pattern algorithm with the Ridom StaphType version 1·5 software package. The default settings recommended by the manufacturer were used.

Statistical analysis

To control for the known effects of age and sex on the prevalence of *S aureus* nasal carriage [1] and the possible clustering of *S aureus* carriage at a family practice level, we calculated the *S aureus* prevalence for each country with a multilevel logistic regression model. This model had three levels (country, family doctor, and patient) and estimated the country prevalence of *S aureus* on the basis of the age and sex sample structure (the total sample population). The multilevel analysis was done with the software package MLwiN. [14]
Because UK participants had a minimum age of 18 years, compared with 4 years in the other participating countries, the adjusted *S aureus* prevalence was calculated twice: first, with all participants of all ages (excluding UK participants) to provide the age and sex sample structure, and second, with all study participants aged 18 years or older (including UK participants).
For calculations of MRSA prevalence, both the number of isolated *S aureus* strains and the total study population were used as denominators. [15]
Multidrug resistance was defined as a strain being resistant to three or more antibiotic classes.[16] For this reason, azithromycin, erythromycin, and clindamycin were grouped together in the calculation of the number of antibiotic classes to which *S aureus* was resistant.
PASW version 18·0 was used for the statistical analyses; a p value of less than 0·05 was defined as significant.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Table 1 shows the baseline characteristics of the APRES patient population. The number of patients recruited per country ranged from 3132 (Belgium) to 4017 (Hungary), constituting a total of 32 770 patients (table 1). Patients who did not meet the age inclusion criterion (people aged <18 years in the UK or <4 years in the other participating countries), incompleteness of data (missing patient background or laboratory data), or incorrect sampling (no bacterial growth recorded at laboratory analysis) was noted in 564 cases (1·7% [95% CI 1·6–1·9]; range 0·3% [0·2–0·5] in

France and Spain to 4·2% [3·7–4·9] in Hungary; table 1). Thus, 32 206 patients were included for analysis.

[TABLE 1]

The overall crude prevalence of *S aureus* nasal carriage was 21·6% (n=6956). Older age was associated with low *S aureus* prevalence (overall odds ratio [OR] 0·992, 95% CI 0·991–0·993). For more data about prevalence in different age groups, see appendix. Additionally, men had a higher chance of nasal carriage of *S aureus* (overall OR 1·38, 1·31–1·46) than did women. The adjusted *S aureus* prevalence for patients older than 18 years was highest in Sweden (29·4%) and lowest in Hungary (12·1%; table 2).

[TABLE 2]

Antibiotic resistance results were available for 6908 (99·3%) of 6956 *S aureus* isolates. Resistance to penicillin was most common (5056 [73·2%] of 6908). Apart from penicillin, macrolide (azithromycin and erythromycin) and clindamycin resistance had the greatest variability between countries, with the highest resistance in France (16·8%) and Belgium (14·6%), and the lowest in Sweden for both macrolides (1·6%) and clindamycin (1·5%; table 3). Antibiotic resistance was less than 10% for all other drugs tested in all participating countries (table 3).

[TABLE 3]

When the MIC distribution of azithromycin was studied, all countries showed a peak at MIC of 1 mg/L, and more than 78% of azithromycin-resistant *S aureus* were highly resistant (MIC ≥32 mg/L) to this drug in all countries.
Multidrug resistance was most common in the UK (3·0%, n=24) and least common in Sweden (0·2%, n=2; table 4). Six isolates were resistant to five antibiotic classes (one isolate in Belgium, one in Croatia, two in the Netherlands, and two in the UK) and one *S aureus* isolate was resistant to six classes (Croatia).

[TABLE 4]

In total, 91 MRSA isolates were identified, with the prevalence ranging from no isolates in Sweden to 16 in France (table 5). When the number of isolated *S aureus* was used as denominator in calculations of MRSA prevalence, Belgium had the highest prevalence (2·1%; table 5). Based on the total study population per country, Belgium, Croatia, France, and the UK had the highest MRSA prevalence (0·4%; table 5). There was no evidence to suggest clustering of MRSA strains in any family doctor practice; the only occurrence was two Dutch MRSA strains with *spa* type t002 that originated from patients from the same practice (table 5). All other similar *spa* types per country belonged to patients from different practices.

[TABLE 5]

53 different *spa* types and two non-typeable strains were recorded in the 91 MRSA isolates (table 5). *Spa* type t002 was the type most commonly identified in MRSA isolates (n=9), followed by t008 (n=7). 70 (77%) of 91 strains could be clustered into eight *spa* clonal complexes, 16 were classified as singletons, and three strains were excluded because the *spa* types identified were less than five repeats in length (figure). All MRSA strains belonging to *spa* clonal complex 011 (n=10) were resistant to tetracycline, compared with 17·3% (14 of 81) of the non-*spa*-clonal complex 011 MRSA (OR 1·71, 95% CI 1·22–2·40).

[FIGURE 1]

DISCUSSION

This study included samples from more than 32 000 patients who did not have traditional risk factors for antibiotic resistance (an infectious disease at inclusion, recent antibiotic use, or recent admission to hospital) and is the first study in which standardised methods have been used to compare the prevalence and antibiotic resistance of commensal *S aureus* within and between countries (panel). The use of a central microbiological laboratory to do the antibiotic resistance analyses removes interlaboratory bias. The participation of nationwide family doctor networks, together with the relatively large study population, enables accurate estimates of the levels of resistance in commensal *S aureus* in every country in patients visiting family doctor practices for a non-infectious disorder.

[BOX 1]

Only the anterior nares were used as a sampling site, which could lead to underestimation of the true prevalence of *S aureus* and MRSA carriage, because the pharynx, skin, and perineum are also common habitats of commensal *S aureus*. [1] However, to keep this large surveillance study feasible, we chose to limit data collection to nasal swabs.

The prevalence of *S aureus* carriage might have been affected by interlaboratory differences, although we have reduced these differences by the use of protocols for the isolation and identification of *S aureus* and with a validation study before the start of this investigation. [9]

S aureus nasal carriage in healthy adult populations has previously been suggested to be about 27%, although several recent studies have reported a prevalence of about 20%, which is similar to our results. [1, 20 and 21] In this study, a wide range in nasal *S aureus* carriage was noted between the participating countries, with participants in Sweden having a prevalence more than double that of Hungarian participants (29·4% *vs* 12·1%), even after controlling for age, sex, and family doctor. Whether actual differences in *S aureus* nasal carriage exist between the participating countries is difficult to assess with the present study design, because genetic factors have been reported to contribute to the *S aureus* colonisation status of an individual and these factors were not measured here. [22] However, in view of the extent of the intercountry variations, future studies of *S aureus* nasal carriage might explore reasons for these differences.

The prevalence of antibiotic resistance was low in the isolated *S aureus*, with no resistance detected for linezolid and vancomycin. Furthermore, resistance was less than 3% for gentamicin and co-trimoxazole, and ciprofloxacin and tetracycline resistance did not exceed 8% in any participating country. However, three isolates were resistant to daptomycin. The restricted use of this drug in clinical practice makes it remarkable that resistance was identified in healthy people in the outpatient setting. [23]

Apart from the well known high prevalence of penicillin resistance, the highest resistance and largest intercountry variation was reported for the macrolides. The relatively high extramural use of these drugs in Gram-positive infections could be an explanation for this result.[24] The situation is especially serious in France and Belgium, where azithromycin and erythromycin resistance rates approach 20%. These rates of resistance in commensal *S aureus* create the question of whether macrolides should still be prescribed empirically for putative *S aureus* infections in these countries. Apart from penicillin and tetracycline, the Swedish commensal *S aureus* had the lowest prevalence of resistance to all antibiotics tested by comparison with the other participating countries. This result was further exemplified by no cases of MRSA being isolated in Sweden, and the discovery of only two multidrug-resistant *S aureus* strains there (0·2% of all Swedish *S aureus* strains). These data are partly consistent with data for outpatient antibiotic use from the European Surveillance of Antibiotic Consumption, which for example show high macrolide use in France. [17] However, the high ciprofloxacin resistance recorded in UK *S aureus* strains is notable, because UK fluoroquinolone use was reportedly the lowest in Europe. [17] In the APRES project, antibiotic use in the participating family doctor networks is being investigated and the relation between use and resistance will possibly explain the results presented here.

The low MRSA prevalence noted in this report, which did not exceed 2·1% in any country, contrasts with the data from *S aureus* strains causing bloodstream infections, in which the MRSA prevalence ranged from 0·5% in Sweden to 30·2% in Hungary, when only countries participating in APRES are taken into account. [4] The low prevalence of MRSA in healthy people has been reported previously in several countries. [19 and 25] The continuing low MRSA prevalence in this population could be the result of increased public awareness of MRSA and subsequent public health measures taken to control MRSA in the past few years.

The population structure of the MRSA strains showed great heterogeneity, with 53 unique *spa* types identified in the 91 MRSA strains. When these were clustered into *spa* clonal complexes, much heterogeneity remained, although in a few countries one cluster predominated. In France, more than half of the MRSA belonged to *spa* clonal complex 008, which corresponds to MRSA clonal complex 8, and more than 60% of the UK MRSA strains were clustered in *spa* clonal complex 022/032 (MRSA clonal complex 22; EMRSA-15). Grundmann and colleagues, [26] who studied the geographical distribution of dominant invasive *S aureus* in Europe, reported *spa* types t008 and t032 to be unexpectedly common in France and the UK, respectively. In the Netherlands, more than 50% of the MRSA were grouped in *spa* clonal complex 011, which included livestock-associated *spa* types. [27] The increase in the proportion of MRSA associated with livestock in the Netherlands had been reported previously. [27 and 28]

All MRSA strains within *spa* clonal complex 011 showed resistance to tetracycline, which has been implicated as a good initial marker for MRSA CC398. [29] The high tetracycline use in the veterinary sector could provide a rationale for this association. The genetic diversity noted for MRSA strains contrasts with the clustering of invasive MRSA strains.[26] In a European multicentre study,[30] a convenience sample of 568 *S aureus* strains (infectious and commensal) isolated during a 10 year period from patients attending a health-care centre or hospital also revealed genotypic diversity. Together with the low resistance reported in this study, this diversity implies that most *S aureus* resistance genes do not expand clonally or spread easily in the community, but once inside a health-care setting they can transfer from one host to another. This finding contrasts with a French study by Dufour and colleagues, [31] who reviewed 14 patients with community-acquired MRSA infections in 2002 and concluded that a super-adapted MRSA strain seemed to be spreading in the community. To control the amounts of MRSA in the extramural setting, continuous surveillance and translation of the results into guidelines for family doctors are of the utmost importance.

In conclusion, in healthy people recruited from family doctor practices in nine European countries with different amounts and patterns of antibiotic prescription behaviour, a low prevalence of MRSA was identified. MRSA strains showed a notable genotypic heterogeneity within and between countries, suggesting low spread of MRSA in the community. Additionally, the prevalence of *S aureus* nasal carriage varied substantially by country and the rates of antibiotic resistance were low, with the exception of penicillins (all countries) and the macrolides (France and Belgium).

Contributors

WJP, MP, HG, FGS, and EES designed and wrote the study protocols. CDJdH, EMEvB, WJP, CAB, FGS, and EES coordinated the study. Members of the APRES study team were responsible for the recruitment of patients and processing of the nasal swabs in their country of residence. CDJdH and EES wrote the first and final drafts. All authors contributed to the writing of the report.

APRES study team

Medical University of Vienna, Austria: Kathryn Hoffmann. *Elisabethinen Hospital, Linz, Austria:* Petra Apfalter. *Catholic University of Leuven, Leuven, Belgium:* Stefaan Bartholomeeusen. *University of Antwerp, Antwerp, Belgium:* Greet Ieven. *University of Zagreb, Zagreb, Croatia:* Milica Katic. *University Hospital Centre, Zagreb, Croatia:* Ana Budimir. *French Society of General Practice, Issy-les-Moulineaux, France:* Gilles Hebbrecht. *University of Debrecen, Debrecen, Hungary:* László Kolozsvári, Jozsef Konya. *The Netherlands Institute for Health Services Research, Utrecht, The Netherlands:* Joke Korevaar. *Institut Universitari d'Investigació en Atenció Primària Jordi Gol, Barcelona, Spain:* Bonaventura Bolibar. *Institut Català de la Salut Metropolitana Sud, l'Hospitalet, Spain:* Elisabet Grenzner. *Lund University, Malmö, Sweden:* Sigvard Mölstad. *Ryhov County Hospital, Jönköping, Sweden:* Andreas Matussek. *Royal College of General Practitioners, Birmingham, UK:* Douglas Fleming. *North Bristol NHS Trust, Bristol, UK:* Andrew Lovering.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

This study was funded by the European Commission Directorate-General for Research within its 7th Framework Programme(grant agreement 223083). We thank all laboratory personnel, participating general practitioners, and their patients, without whose help we could not have done this study. We remember Helmut Mittermayer, who was the initiator of the Austrian laboratory participation in APRES, but sadly passed away mid-2010. Additionally, we thank Stefan Visscher for all the work he has put into the collection of the Dutch data; Christel Driessen, Jacqueline Maes, Jacqueline Peeters, Mayk Lucchesi, Resi Hoogenboom, and Bram Vanderzanden who did all laboratory diagnostics at the project's central microbiological laboratory; Samuel Coenen for his help with the data analysis; Peter Spreeuwenberg for doing the multilevel analysis; and the rest of the research partner teams.

REFERENCES

1 HF Wertheim, DC Melles, MC Vos, *et al.* The role of nasal carriage in *Staphylococcus aureus* infections Lancet Infect Dis, 5 (2005), pp. 751–762
2 J Kluytmans, A van Belkum, H Verbrugh Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks Clin Microbiol Rev, 10 (1997), pp. 505–520
3 GA Donker, RH Deurenberg, C Driessen, S Sebastian, S Nys, EE Stobberingh The population structure of *Staphylococcus aureus* among general practice patients from The Netherlands Clin Microbiol Infect, 15 (2009), pp. 137–143
4 European Centre for Disease Prevention and Control (ECDC) Annual Surveillance Report. Reporting on 2009 surveillance data and 2010 epidemic intelligence dataECDC, Stockholm (2011) http://www.ecdc.europa.eu.proxy.library.uu.nl/en/publications/Publications/1111_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf (accessed Jan 4, 2013).
5 H Goossens, M Ferech, R Vander Stichele, M Elseviers Outpatient antibiotic use in Europe and association with resistance: a cross-national database study Lancet, 365 (2005), pp. 579–587
6 A Kronenberg, S Koenig, S Droz, K Muhlemann Active surveillance of antibiotic resistance prevalence in urinary tract and skin infections in the outpatient setting Clin Microbiol Infect, 17 (2011), pp. 1845–1851
7 V de Lastours, F Chau, F Tubach, B Pasquet, E Ruppe, B Fantin Independent behavior of commensal flora for carriage of fluoroquinolone-resistant bacteria in patients at admission Antimicrob Agents Chemother, 54 (2010), pp. 5193–5200
8 A Andremont, M Bonten, J Kluytmans, Y Carmeli, O Cars, S Harbarth Fighting bacterial resistance at the root: need for adapted EMEA guidelines Lancet Infect Dis, 11 (2011), pp. 6–8
9 EM van Bijnen, CD den Heijer, WJ Paget, *et al.* The appropriateness of prescribing antibiotics in the community in Europe: study design BMC Infect Dis, 11 (2011), p. 293
10 European Committee on Antimicrobial Susceptibility Testing EUCAST Definitive Document E.DEF 3.1, June 2000: determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution Clin Microbiol Infect, 6 (2000), pp. 509–515

11 CD Steward, PM Raney, AK Morrell, *et al.* Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus* J Clin Microbiol, 43 (2005), pp. 1716–1721
12 C Ryffel, W Tesch, I Birch-Machin, *et al.* Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*
Gene, 94 (1990), pp. 137–138
13 B Strommenger, C Kettlitz, T Weniger, D Harmsen, AW Friedrich, W Witte Assignment of *Staphylococcus* isolates to groups by spa typing, SmaI macrorestriction analysis, and multilocus sequence typing J Clin Microbiol, 44 (2006), pp. 2533–2540
14 Rasbash J, Charlton C, Browne WJ, Healy M, Cameron B. MLwiN Version 2.1, Centre for Multilevel Modelling, University of Bristol. 2009.
15 M Dulon, F Haamann, C Peters, A Schablon, A Nienhaus MRSA prevalence in European healthcare settings: a review BMC Infect Dis, 11 (2011), p. 138
16 AP Magiorakos, A Srinivasan, RB Carey, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance Clin Microbiol Infect, 18 (2012), pp. 268–281
17 N Adriaenssens, S Coenen, A Versporten, *et al.* European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe (1997–2009)
J Antimicrob Chemother, 66 (suppl 6) (2011), pp. vi3–v12
18 C Costelloe, A Lovering, A Montgomery, D Lewis, C McNulty, AD Hay Effect of antibiotic prescribing in primary care on meticillin-resistant *Staphylococcus aureus* carriage in community-resident adults: a controlled observational study Int J Antimicrob Agents, 39 (2012), pp. 135–141
19 R Sá-Leão, IS Sanches, I Couto, CR Alves, H de Lencastre Low prevalence of methicillin-resistant strains among *Staphylococcus aureus* colonizing young and healthy members of the community in Portugal Microb Drug Resist, 7 (2001), pp. 237–245
20 LG Bode, JA Kluytmans, HF Wertheim, *et al.* Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus* N Engl J Med, 362 (2010), pp. 9–17
21 CS Chen, CY Chen, YC Huang Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university Int J Infect Dis, 6 (2012), pp. e799–e803
22 M Johannessen, JE Sollid, AM Hanssen Host- and microbe determinants that may influence the success of *S aureus* colonization Front Cell Infect Microbiol, 2 (2012), p. 56
23 PA Moise, D North, JN Steenbergen, G Sakoulas Susceptibility relationship between vancomycin and daptomycin in *Staphylococcus aureus*: facts and assumptions Lancet Infect Dis, 9 (2009), pp. 617–624
24 R Leclercq Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications Clin Infect Dis, 34 (2002), pp. 482–492
25 C Lozano, E Gomez-Sanz, D Benito, C Aspiroz, M Zarazaga, C Torres *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains Int J Med Microbiol, 301 (2011), pp. 500–505
26 H Grundmann, DM Aanensen, CC van den Wijngaard, BG Spratt, D Harmsen, AW Friedrich Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis PLoS Med, 7 (2010), p. e1000215
27 BA van Cleef, DL Monnet, A Voss, *et al.* Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe Emerg Infect Dis, 17 (2011), pp. 502–505
28 MM van Rijen, PH Van Keulen, JA Kluytmans Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming Clin Infect Dis, 46 (2008), pp. 261–263
29 C Lozano, A Rezusta, P Gomez, *et al.* High prevalence of spa types associated with the clonal lineage CC398 among tetracycline-resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital J Antimicrob Chemother, 67 (2012), pp. 330–334
30 J Rolo, M Miragaia, A Turlej-Rogacka, *et al.* High genetic diversity among community-associated *Staphylococcus aureus* in Europe: results from a multicenter study PLoS One, 7 (2012), p. e34768

31 P Dufour, Y Gillet, M Bes, *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton–Valentine leucocidin Clin Infect Dis, 35 (2002), pp. 819–824

TABLE

Table 1. Baseline characteristics of the participating patients

	Total patient population (n)	Excluded from analysis (n)			Women (% of total)	Age distribution (% of total)		
		Age*	Mismatch†	Incorrect sampling‡		4–19 years	20–64 years	≥65 years
Austria	3380	4	56	11	56·6%	5·3%	72·3%	22·4%
Belgium	3132	6	101	0	54·2%	5·2%	57·9%	37·0%
Croatia	4013	1	35	17	59·4%	16·0%	53·9%	30·1%
France	3870	0	12	0	55·0%	9·5%	64·6%	25·9%
Hungary	4017	3	17	150	55·0%	28·2%	50·4%	21·3%
Netherlands	3873	14	12	0	57·1%	10·3%	63·8%	25·9%
Spain	4001	0	11	0	58·6%	12·0%	57·2%	30·8%
Sweden	3273	33	26	0	57·4%	12·6%	54·9%	32·5%
UK	3211	3	52	0	59·2%	1·6%	69·1%	29·3%
Total	32 770	64	322	178	57·0%	11·6%	60·3%	28·2%

* Number of patients who did not meet the age inclusion criterion—ie, aged <18 years in the UK and <4 years in the other participating countries.
† Defined as the absence of patient background information or laboratory data.
‡ Defined as the absence of bacteria (including normal nasal microbiota) on the agar plate at laboratory analysis.

Table 2. Unadjusted and adjusted *Staphylococcus aureus* prevalence by country

	Swabs tested (n)	**Prevalence of *S aureus* nasal carriage (% [95% CI])**		
		Unadjusted	**Adjusted* (all ages†)**	**Adjusted* (aged ≥18 years only‡)**
Austria	3309	16·6% (15·4–17·9)	16·2% (13·2–19·8)	15·7% (12·7–19·2)
Belgium	3025	19·3% (17·9–20·8)	19·4% (15·9–23·4)	18·8% (15·3–22·9)
Croatia	3960	20·0% (18·8–21·3)	19·4% (16·0–23·3)	18·5% (15·1–22·5)
France	3858	22·7% (21·4–24·0)	21·9% (18·2–26·2)	21·1% (17·4–25·4)
Hungary	3847	14·1% (13·0–15·2)	12·7% (10·3–15·6)	12·1% (9·7–15·1)
Netherlands	3847	27·9% (26·5–29·3)	27·3% (22·9–32·1)	26·3% (22·0–31·3)
Spain	3990	19·3% (18·2–20·6)	18·8% (15·6–22·6)	17·3% (14·2–21·0)
Sweden	3214	29·8% (28·2–31·4)	29·4% (24·7–34·5)	29·4% (24·6–34·8)
UK	3156	25·8% (24·3–27·3)	..§	25·4% (21·0–30·3)

* Multilevel logistic regression model, which adjusted for age, sex, and family doctor.
† All participants of any age (excluding UK participants) were used as the standard population.
‡ All participants aged ≥18 years (including UK participants) were used as the standard population.
§ Not applicable to the UK since no patients younger than 18 years were included because of ethics committee constraints.

Table 3. Lowest and highest prevalence of antibiotic resistance of commensal *Staphylococcus aureus*

	Lowest antimicrobial resistance			**Highest antimicrobial resistance**		
	Country	**Prevalence (% [95% CI])**	**Antibiotic use* (rank)**	**Country**	**Prevalence (% [95% CI])**	**Antibiotic use* (rank)**
Azithromycin	Sweden	1·6% (1·0–2·6)	0·63 (1)	France	16·9% (14·6–19·6)	4·15 (9)
Ciprofloxacin	Sweden	0·6% (0·3–1·4)	0·79 (2)	UK	5·2% (3·8–6·9)	0·48 (1)
Clindamycin	Sweden	1·5% (0·9–2·4)	0·63 (1)	Belgium	14·6% (12·0–17·7)	2·96 (5)
Erythromycin	Sweden	1·6% (1·0–2·6)	0·63 (1)	France	16·5% (14·2–19·1)	4·15 (9)
Gentamicin	Sweden, France	0·0% (0·0–0·4)	..†	Austria	2·2% (1·3–3·8)	..†
Penicillin	Austria	64·8% (60·8–68·7)	7·09 (4)	Spain	87·1% (84·6–89·3)	12·31 (7)
Tetracycline	Spain	1·8% (1·1–3·0)	0·60 (1)	Croatia	7·2% (5·5–9·2)	1·57 (4)
Co-trimoxazole	Sweden	0·0 % (0·0–0·4)	0·54 (5)	UK	1·0% (0·5–1·9)	1·18 (9)

No resistance to linezolid or vancomycin was reported; three isolates were resistant to daptomycin (two from the Netherlands and one from the UK).

* The national outpatient antibiotic use per antibiotic class according to the European Surveillance of Antimicrobial Consumption data, expressed in defined daily dose per 1000 inhabitants and per day.[17] The national outpatient antibiotic use rates of all participating countries are ranked, in which rank 1=country with the lowest antibiotic use, and rank 9=country with the highest antibiotic use.

† The national outpatient use of aminoglycosides is not given by European Surveillance of Antimicrobial Consumption.

Table 4.:Number of antibiotic classes to which *Staphylococcus aureus* was resistant by country

	n	0 classes (%)	1 class (%)	2 classes (%)	≥3 classes (%)*
Austria	549	29·9%	55·7%	12·4%	2·0%
Belgium	582	20·1%	64·9%	12·9%	2·1%
Croatia	755	20·0%	67·8%	9·4%	2·8%
France	874	19·1%	67·4%	11·6%	1·9%
Hungary	539	19.5%	65·7%	12·1%	2·8%
Netherlands	1073	28·5%	61·5%	8·0%	2·0%
Spain	769	10·8%	75·6%	11·6%	2·1%
Sweden	955	33·2%	63·6%	3·0%	0·2%
UK	812	23·0%	63·4%	10·6%	3·0%

* A strain that was resistant to three or more antibiotic classes was classified as multidrug resistant.

Table 5. : Prevalence of MRSA and *spa* types recorded by country

	Total number of cases of MRSA	% of MRSA in all *S aureus* strains (95% CI)	% of MRSA in total study population (95% CI)	*Spa* types recorded
Austria	8	1·5% (0·7–2·8)	0·2% (0·1–0·5)	t003, t005 (n=2), t008, t010, t015, t034, t127
Belgium	12	2·1% (1·2–3·6)	0·4% (0·2–0·7)	t008, t011 (n=2), t038, t062, t231, t447 (n=3), t1923, t2346, t10847
Croatia	15	2·0% (1·2–3·3)	0·4% (0·2–0·6)	t002, t003, t005, t011, t014, t015, t045, t050, t127, t330, t535, t550, t2018, t5933, t10807
France	16	1·8% (1·1-3·0)	0·4% (0·3–0·7)	t002 (n=3), t008 (n=5), t010, t121, t622, t681, t777 (n=2), t2054, t5708
Hungary	8	1·5% (0·8–2·9)	0·2% (0·1–0·4)	t002 (n=2), t032, t127 (n=2), t330 (n=2), t1218

	Total number of cases of MRSA	**% of MRSA in all *S aureus* strains (95% CI)**	**% of MRSA in total study population (95% CI)**	***Spa* types recorded**
Netherlands	9	0·8% (0·4–1·6)	0·2% (0·1–0·4)	t011 (n=2), t034, t038, t108, t267, t740, t1457, t10812
Spain	10	1·3% (0·7–2·4)	0·3% (0·1–0·5)	t002 (n=2), t022, t230, t846, t1081, t1203, t1610, t10814
Sweden	0	0·0% (0·0–0·4)	0·0% (0·0–0·1)	None
UK	13	1·6% (0·9–2·7)	0·4% (0·2–0·7)	t002, t020, t025, t032, t127 (n=2), t223, t852, t1214, t2436, t5414, t7922

MRSA=meticillin-resistant *Staphylococcus aureus.*

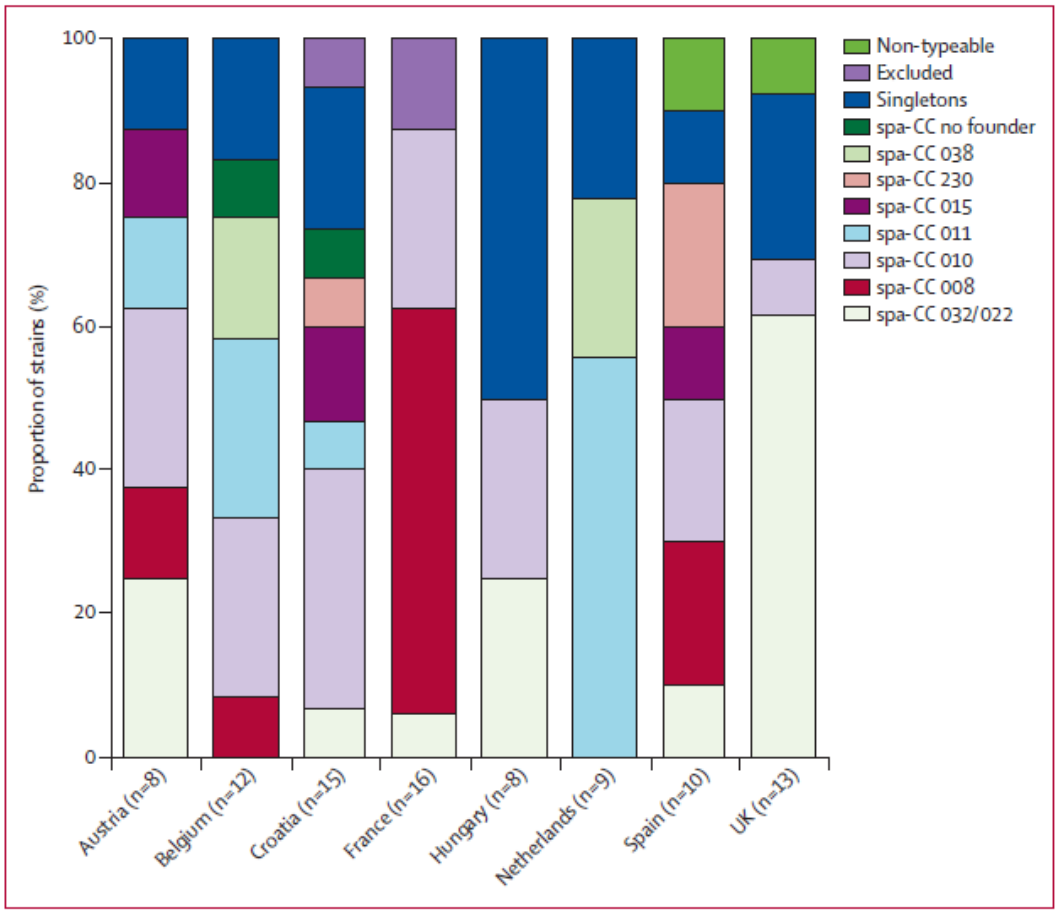

Figure: **Distribution of *spa* clonal complexes by country**
CC=clonal complex. Sweden is not included because no meticillin-resistant *Staphylococcus aureus* was isolated from the Swedish population in this study.

Box

Panel. Research in context

Systematic review

We searched PubMed with the term "*Staphylococcus aureus* AND (healthy OR commensal OR community) AND carriage AND resistance AND Europe" for reports involving human patients, published in English before Jan 3, 2013. We identified 40 publications. Of these results, 23 articles contained data for *Staphylococcus aureus* prevalence or resistance. The other articles were reviews; studies about the composition of the nasopharyngeal microbiota, the interaction of *S aureus* colonisation between animals and human beings, or other staphylococcal species; (exclusively) molecular publications; case reports; or small case series.

Of the 23 articles about *S aureus* prevalence and resistance, five were based on samples from patients with specific diseases (eg, coeliac disease) or within specific health-care settings (hospital or long-term care facilities). In three studies, the *S aureus* strains originated from sites other than the anterior nares, and infectious *S aureus* strains were the focus in seven studies. In one study, the investigators focused primarily on the carriage status of *S aureus* without consideration of the antibiotic resistance pattern of the isolates, and in two studies the investigators reported the prevalence of meticillin-resistant *S aureus* (MRSA) carriage only, without giving information about the *S aureus* prevalence. In the remaining five studies—done in the UK (n=2), Spain, Italy, and Portugal—investigators reported the prevalence of both *S aureus* and MRSA nasal carriage. The prevalence of nasal *S aureus* carriage ranged from 16% in UK resident adults 16 years or older (n=5917) in a controlled observational study by Costelloe and colleagues, [18] to 46% in Portuguese high-school students (n=107) in a cross-sectional study by Sá-Leão and colleagues. [19] Costelloe and colleagues [18] reported the highest prevalence of MRSA (3·3%), whereas all other studies reported an MRSA prevalence of less than 1%. All five studies were done in one country and did not exclude patients with recent antibiotic use or hospital admission, thereby potentially affecting the prevalence and resistance within their collection of *S aureus*.

Interpretation

Our study adds to existing knowledge by giving insight into the geographical variation of the prevalence of commensal *S aureus* carriage; the presence of antibiotic-resistant *S aureus* in asymptomatic carriers, which is an important reservoir of genetic resistance elements in the community; and the molecular epidemiology of asymptomatic MRSA carriage in nine countries in Europe.

THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: den Heijer CDJ, van Bijnen EME, Paget WJ, et al, and the APRES Study Team. Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *S aureus*, in nine European countries: a cross-sectional study. *Lancet Infect Dis* 2013; published online March 6. http://dx.doi.org/10.1016/S1473-3099(13)70036-7.

Table. Age-stratified prevalence of *S. aureus* carriage by country

Country	Swabs tested (n)	Prevalence of *S aureus* nasal carriage (% [95% CI]) by age (years)*		
		4-19	20-64	≥65 years
Austria	3,309	23·9 (18·5–30·3)	17·6 (14·3–21·5)	14·6 (11·6–18·2)
Belgium	3,025	26·8 (20·8–33·8)	21·6 (17·6–26·3)	14·5 (11·7–17·9)
Croatia	3,960	26·7 (22·0–32·1)	20·7 (16·9–25·1)	15·5 (12·6–19·0)
France	3,858	29·9 (24·4–36·0)	23·3 (19·2–28·0)	17·0 (13·8–20·9)
Hungary	3,847	19·9 (16·3–24·2)	13·8 (11·0–17·1)	14·5 (11·5–18·0)
The Netherlands	3,847	36·2 (30·2–42·8)	29·6 (24·8–35·0)	21·1 (17·2–25·5)
Spain	3,990	31·5 (26·2–37·3)	19·4 (15·9–23·5)	14·6 (11·9–17·8)
Sweden	3,214	31·8 (26·1–38·0)	33·0 (27·6–38·8)	25·2 (20·8–30·3)
UK	3,156	25·0 (17·9–33·7)	27·0 (22·3-32·3)	24·0 (19·6–28·9)

CI, confidence interval; UK, United Kingdom.
*Calculated using a multilevel logistic regression model, which adjusted for family doctor, sex and age (within the specified age category).