Eur J Clin Microbiol Infect Dis (2005) 24: 145–148
DOI 10.1007/s10096-004-1258-6

CONCISE ARTICLE

E. Nulens · I. Gould · F. MacKenzie · A. Deplano · B. Cookson · E. Alp · E. Bouza · A. Voss

Staphylococcus aureus carriage among participants at the 13th European Congress of Clinical Microbiology and Infectious Diseases

Published online: 20 January 2005

Abstract The aim of this study was to measure the rate of *Staphylococcus aureus* nasal colonization among attendees of the 13th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), particularly with regard to methicillin-resistant (MRSA) strains. The 31.4% rate of S*taphylococcus aureus* colonization detected among the participants was in line with colonization rates reported previously for healthcare workers. A statistical difference was found between the rates of *Staphylococcus aureus* carriage in physicians (37.4%) and non-physicians (21.7%) but not between males (35.0%) and females (28.9%). Only one participant (a Belgian physician) was found to carry MRSA. Surprisingly, the rate of methicillin-susceptible *Staphylococcus aureus* carriage was significantly higher among participants from countries with a low prevalence of MRSA.

E. Nulens · E. Alp · A. Voss (✉)
Department of Medical Microbiology, Nijmegen University Centre for Infectious Diseases,
University Medical Centre St. Radboud, 440 MMB,
P.O. Box 9101, 6500 Nijmegen, The Netherlands
e-mail: a.voss@mmb.umcn.nl
Tel.: +31-24-3617575
Fax: +31-24-3540216

I. Gould · F. MacKenzie
Department of Medical Microbiology, Aberdeen Royal Infirmary,
Foresterhill, Aberdeen, AB25 2ZN, UK

A. Deplano
Laboratoire de Référence MRSA-Staphylocoques, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles,
route de Lennik 808,
1070 Brussels, Belgium

B. Cookson
Laboratory of Hospital Infection, Central Public Health Laboratory, Public Health Laboratory Service,
61 Colindale Avenue,
London, NW9 5HT, UK

E. Bouza
Department of Clinical Microbiology and Infectious Diseases,
Hospital General Universitario Gregorio Marañón,
46 Dr. Esquerdo,
28007 Madrid, Spain

Introduction

In Europe, methicillin-resistant *Staphylococcus aureus* (MRSA) is heterogeneously spread among in- and outpatients. While most European countries have a high prevalence of MRSA, in Scandinavian and Dutch hospitals MRSA is detected in patients only rarely or not at all [1, 2]. Healthcare workers (HCWs) exposed to patients with MRSA may become transient or permanent MRSA carriers and subsequently serve as a source of further transmission among patients and other HCWs. Therefore, most countries have developed hospital infection control guidelines to prevent the colonization of HCWs from patients with proven and/or suspected MRSA. Whether exposure to MRSA outside the hospital poses a risk to HCWs, or even exists, is not yet known.

In the study presented here, the ESCMID Study Group on Nosocomial Infections (ESGNI) aimed to measure the rate of *Staphylococcus aureus*, especially MRSA, colonization among attendees of the 13th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) that took place in Glasgow in 2003.

Materials and methods

One thousand swabs with liquid Ames transport medium (Becton Dickinson, Sparks, MD, USA) and a short questionnaire were randomly placed in the congress bags given to all ECCMID attendees during congress registration. Individuals who received these materials were asked to fill in the questionnaire, culture their anterior nares with the swab according to the provided guidelines, replace the swab in the transport medium, and deliver the swab and completed questionnaire to a collection point. The questionnaire gathered information on the participant's profession (including the specialty for physicians), patient contact, number of

years in the profession, and the country of current professional practice. All swabs were delivered to one centre (Department of Medical Microbiology, University Medical Centre Nijmegen, The Netherlands) for microbiological examination.

The swabs were placed in nutrient broth no. 2, supplemented with 7% NaCl, adjusted to pH 7.2 (Oxoid, Haarlem, The Netherlands), and incubated at 34°C. After 24 h, the suspension was inoculated onto Columbia blood agar and mannitol salt agar, and incubated at 34°C. The plates were evaluated for growth of *Staphylococcus aureus* after 24 and 48 h of incubation. Colonies suspected to be *Staphylococcus aureus* were initially screened for their ability to agglutinate rabbit plasma. If this test was negative but *Staphylococcus aureus* was still suspected, Staphaurex (Remel, Lenexa, KS, USA) and a tube-coagulase test were performed to exclude *Staphylococcus aureus*.

Susceptibility testing was performed with a disk diffusion assay using a 0.5 McFarland inoculum and 5 µg oxacillin disks on Mueller–Hinton agar supplemented with 2% NaCl. The plates were evaluated after 24 and 48 h of incubation at 34°C. Strains were considered resistant if the inhibition zone was <20 mm and when any growth around the disk was observed. Strains were considered heterogeneously resistant when partial growth within the inhibition zone or microcolonies around the oxacillin disk were observed. In addition, a disk diffusion assay using 5 µg ciprofloxacin disks on Mueller–Hinton agar and an inoculum of 0.5 McFarland was performed [3]. All oxacillin- and ciprofloxacin-resistant strains were tested for the presence of the *mec*A gene according to the method described by Murakami et al. [4].

In the case of MRSA detection, genomic DNA extraction, *Sma*I restriction, and DNA fragment separation by pulsed-field gel electrophoresis were performed as described before [5].

Results and discussion

Close to 5,000 delegates from 87 different countries attended the ECCMID in 2003. Of the 1,000 randomly distributed swabs, 335 (33.5%) were returned by attendees from 49 different countries. The distribution of the congress attendees and the study participants from different countries is displayed in Table 1

For six (1.8%) samples the sex of the participant was not available, but none of these volunteers was a *Staphylococcus aureus* carrier. For the remaining samples,

Table 1 Distribution by country of ECCMID attendees, study participants (for countries with >2 participants in the study), and *S. aureus* carriers per country

Country	No. of ECCMID attendees	No. (%) of study participants[a]	No. (%) of *S. aureus* carriers[b]
Great Britain	542	46 (8.5)	12 (26.1)
The Netherlands	144	45 (31.3)	20 (44.4)
Belgium	187	34 (18.2)	14 (41.2)
Sweden	102	23 (22.6)	8 (34.8)
Greece	235	20 (8.5)	2 (10.0)
Switzerland	107	18 (16.8)	6 (33.3)
USA	270	13 (4.8)	2 (15.4)
Germany	201	11 (5.5)	6 (54.5)
Romania	28	11 (39.3)	1 (9.1)
Finland	70	10 (14.3)	5 (50.0)
Denmark	58	8 (13.8)	0
Italy	166	8 (4.8)	2
Slovenia	33	7 (21.2)	3
Norway	46	6 (13.0)	3
Slovakia	17	6 (35.3)	1
Poland	49	6 (12.3)	1
Spain	201	5 (2.5)	3
NA	NA	5 (ND)	0
Canada	40	4 (10.0)	2
Russia	47	3 (6.4)	2
France	160	3 (1.9)	0
Czech Republic	59	3 (5.1)	0
Serbia	9	3 (33.3)	1
Others (2 participants)	455	16 (3.5)	6
Others (1 participant)	254	21 (8.3)	5

[a]Percentage of respective ECCMID attendees (= measure of willingness to participate in study)

[b]Percentages provided for countries represented by 10 or more participants

NA, not available

Table 2 Rates of MSSA colonization among participants from countries with low and high prevalences of MRSA

Country[a]	No. of ECCMID attendees	No. (%) of study participants	No. (%) of *S. aureus* carriers
Low-prevalence countries			
The Netherlands	144	45	20
Sweden	102	23	8
Finland	70	10	5
Denmark	58	8	0
Norway	46	6	3
Total	420	92 (21.9%)	36 (39.1%)
Other countries	2,244	188 (8.4%)	52 (27.6%)

[a]Only countries with >2 study participants were included in this evaluation

163 (49.5%) were from male, and 166 (50.5%) from female volunteers. Of the 335 swabs, 105 (31.4%) were culture-positive for *Staphylococcus aureus*, with 28.9% of the carriers being female and 35% male ($P>0.05$). Table 1 shows the distribution of the *Staphylococcus aureus* carriers per country, with a percentage provided for countries with at least 10 participants.

Among the 214 physicians who participated in the study, 80 (37.4%) were *Staphylococcus aureus* carriers, while only 25 (21.7%) of the 115 non-physicians were *Staphylococcus aureus* carriers ($P<0.05$). About 80% of the physicians had regular contact with patients, with more than half of them reporting contact on a daily basis.

The susceptibility screening methods identified nine strains as intermediately susceptible ($n=7$) or resistant ($n=2$) to ciprofloxacin. No heteroresistance to oxacillin was detected, but one patient's swab grew two morphologically different *Staphylococcus aureus* strains resistant to oxacillin and ciprofloxacin. All of the other strains that were intermediately susceptible and resistant to ciprofloxacin were negative for the *mecA* gene.

The only MRSA-positive attendee we could identify was a male Belgian physician, who reported more than 10 years of medical practice and daily patient contact. Two MRSA strains were isolated from this patient's sample, and the strains differed from each other with regard to both their susceptibility patterns and their genotypes. One MRSA strain belonged to PFGE genotype A20, the other to genotype B2; both of these genotypes have been widespread in Belgian hospitals during recent years.

Overall, the rate of MRSA carriage among the screened participants was 0.3%. The rate of *Staphylococcus aureus* carriage in countries with low and high rates of MRSA prevalence was 39.1% and 27.6%, respectively. Furthermore, the willingness to participate in the study seemed to differ according to prevalence of MRSA in the attendee's country; 27.6% of the participants resided in countries with a low prevalence and 8.4% were from countries with a high prevalence.

The aim of this study was to measure the rate of colonization with *Staphylococcus aureus* and, specifically, MRSA colonization among HCWs who participated at the 13th ECCMID. The overall rate of *Staphylococcus aureus* carriage was 31.4%, which is in the range of previously published carriage rates among HCWs [6]. As expected, physicians with patient contact were found to carry *Staphylococcus aureus* significantly more frequently than participants without patient contact.

The finding of a single MRSA carrier among the participants was much lower than expected. This could be explained by the fact that the number of participants from countries known to have a high prevalence of MRSA, such as France, Spain, Portugal and Italy, was very low, which could indicate that participation in this study was negatively correlated with the prevalence of MRSA in the home country of the potential participant. Since the swabs were distributed randomly in the congress bags, we have to assume that ECCMID participants from all countries had an equal opportunity to participate in the study.

When comparing the results it was remarkable that participants from countries known to have a low prevalence of MRSA, such as The Netherlands and Scandinavian countries [2], had the highest rates of *Staphylococcus aureus* carriage (34.8–44.4%), while the rates were clearly lower among participants from the other countries (Table 2). Apparently, *Staphylococcus aureus* carrier status does not necessarily predispose HCWs to MRSA colonization. In fact, carriers of *Staphylococcus aureus* might even be protected from colonization with another (possibly methicillin-resistant) strain, as we know from therapeutic studies with avirulent *Staphylococcus aureus* strains in the pre-antibiotic area [7].

High-level oxacillin resistance and ciprofloxacin resistance was detected in two strains from the same participant using our susceptibility testing method. No heteroresistance was detected in the other strains. Recent reports have indicated that *mec*A-positive heteroresistant strains with relatively low minimum inhibitory concentrations to oxacillin have been found to be associated with resistance to ciprofloxacin in 95% of strains [8, 9]. We consequently examined all *Staphylococcus aureus* strains that were intermediately resistant or resistant to ciprofloxacin for the presence of the *mec*A gene. Of the ten ciprofloxacin intermediate or resistant *Staphylococcus aureus* strains detected, only two were found to have the *mec*A gene. Both of these strains were homogeneously oxacillin resistant and thus would have been detected without screening for ciprofloxacin resistance. Moreover, the two MRSA strains both demonstrated high-level oxacillin resistance. The strains belonged to PFGE genotype A20c and B2y (subtypes from clone A20 and B2, respectively), which

have been prevalent in Belgian hospitals since 2001 and are disseminated in surrounding countries as well [1, 5, 10]. It is unknown whether this physician posed a risk for transmitting MRSA to other participants during social contact at the congress. Since samples were only taken at registration, the study was not equipped to measure possible transmission during the meeting.

Future studies on the subject of *Staphylococcus aureus* carriage among HCWs should further evaluate the possible protective effect of methicillin-susceptible *Staphylococcus aureus* carriage on preventing MRSA carriage. To investigate the possibility of transmission during social contact, future studies should be planned in low-endemic situations that allow easy tracking of MRSA since the results would not be confounded by the presence of multiple types of MRSA. Such studies should include much higher numbers of participants.

References

1. Cookson BD (2000) Methicillin-resistant *Staphylococcus aureus* in the community: new battlefronts, or are the battles lost?. Infect Control Hosp Epidemiol 21:398–403
2. Veldhuizen I, Bronzwaer S, Degener J, Kool J, and EARSS participants (2000) European antimicrobial resistance surveillance system (EARSS): susceptibility testing of invasive *Staphylococcus aureus*. Eurosurveillance 5:34–36
3. National Committee for Clinical Laboratory Standards (1997) Performance standards for antimicrobial susceptibility tests, 6th edn. Approved standard M2-A6 NCCLS, Wayne, PA
4. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S (1991) Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 29:2240–2244
5. Deplano A, Witte W, Van Leeuwen WJ, Brun Y, Struelens MJ (2000) Clonal dissemination of epidemic methicillin-resistant *Staphylococcus aureus* in Belgium and neighboring countries. Clin Microbiol Infect 6:239–245
6. Kluytmans J,Van Belkum A, Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10:505–520
7. Peacock SJ, de Silva I, Lowy FD (2001) What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol 9:605–610
8. Schmitz F-J, Fluit AC, Hafner D, Beeck A, Perdikouli M, Boos M, Scheuring S, Verhoef J, Köhrer K, Von Eiff C (2000) Development of resistance to ciprofloxacin, rifampin, and mupirocin in methicillin-susceptible and -resistant *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 44:3229–3231
9. Wannet W (2002) Spread of an MRSA clone with heteroresistance to oxacillin in the Netherlands. Eurosurveillance 7:73–74
10. Denis O, Deplano A, De Ryck R, Nonhoff C, Struelens MJ (2003) Emergence and spread of gentamicin-susceptible strains of methicillin-resistant *Staphylococcus aureus* in Belgian hospitals. Microb Drug Resist 9:61–71