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SHORT REPORT
Staphylococcus aureus nasal carriage in the community: a survey from central Italy

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SUMMARY

Recently, concern has increased regarding the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in the community. We studied 812 subjects from central Italy to establish the rates of nasal carriage of *S. aureus*, and antibiotic susceptibility patterns, in the community. The prevalence of *S. aureus* nasal carriage was 30·5%. Only one subject, with predisposing risk factors for acquisition, was identified as carrier of MRSA (prevalence of 0·12%). The presence of MRSA in the community of our area still appears to be a rare event. Among methicillin-susceptible *S. aureus* (MSSA) isolates, a surprisingly high rate (18%) of resistance to rifampin was observed.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of nosocomial infection [1]. Risk factors identified for MRSA infection include prolonged hospitalization, invasive or surgical procedures, prolonged or recurrent exposure to antibiotics, chronic diseases, and contact with health-care facilities [2].

In the past, community-acquired MRSA infections have rarely been described, and those that have are mostly in adult patients [3]. Recently, however, there have been an increasing number of reports of community-acquired MRSA infections that involved people without known predisposing risk factors for acquisition [1, 2, 4, 5].

The extent of MRSA carriage within the community is largely unknown, and it varies in different regions [1, 6]. Few data are available for Italy [7]. In this work, we studied the prevalence of MRSA nasal carriage in a sample population from central Italy.

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During a 5-month period (November 2000–March 2001), we obtained samples from 812 subjects. Among these, 398 (62·8% female) ranging in age from 16 to 83 years (mean age 43 years; S.D. = 16), came from the general population that attended the Siena University Hospital for voluntary check-ups. After obtaining informed consent, nasal swabs were taken from anterior nares and patients were interviewed regarding risk factors for MRSA acquisition [2]. The other 414 subjects came from the school community. Among these, 374 were children (51·1% female) ranging in age from 3 to 11 years (mean age 6; S.D. = 2) who attended three nurseries and one primary school; nasal swabs were taken from these subjects at school, after their parents had given informed consent. Notices regarding risk factors for MRSA acquisition were not available for these subjects. The study was also carried out on 40 school guardians (85% female) who ranged in age from 26 to 57 years (mean age 42; S.D. = 11).

Nasal swabs were inoculated directly onto mannitol salt agar (Oxoid). Plates were incubated at 37 °C for

Table 1. *Difference in carriage of* S. aureus *among studied populations*

	School community (414 subjects) (%)	General population (398 subjects) (%)	*P**
S. aureus carriers	145 (35)	103 (25·9)	0·006
Multi-resistant *S. aureus* carriers	50 (12·1)	22 (5·5)	0·002

* Yates corrected χ^2 test.

48 h. Mannitol-fermenting colonies were subcultured onto Columbia agar with 5% of sheep blood (Oxoid) overnight at 37 °C. Presumptive identification was performed on the basis of colony morphology, catalase reaction and Gram stain; isolates were confirmed to be *S. aureus* using a latex agglutination test (Dryspot Staphytect Plus, Oxoid).

All isolates were screened for methicillin-resistance on Mueller–Hinton agar with 4% NaCl and 6 μg/ml oxacillin (Oxacillin Screen Agar, Becton–Dickinson) at 30 °C. Susceptibility testing was performed by disk diffusion, according to National Committee for Clinical Laboratory Standards guidelines [8]. Reference strain used for quality control was *S. aureus* ATCC 25923. All isolates were screened for susceptibility to penicillin, gentamicin, tobramycin, kanamycin [9], erythromycin, clindamycin, ciprofloxacin, vancomycin, teicoplanin, rifampin, chloramphenicol, tetracycline, and trimethoprim–sulphamethoxazole. Susceptibility testing of MRSA isolate was also performed by E-test method (AB Biodisk) for teicoplanin and vancomycin.

In the oxacillin-resistant isolate, the presence of the *mecA* gene was confirmed by polymerase chain reaction as described previously [10], using primers MecA-F (5′-GGGATCATAGCGTCATTATTC) and MecA-R (5′-AACGATTGTGACACGATAGCC); the amplification reaction was carried out in a 50 μl volume, using 2·5 U of *Taq* DNA polymerase (Promega) in the reaction buffer provided by the manufacturer containing 200 μM deoxynucleoside triphosphate and 30 pmol of each primer. After a denaturation step of 5 min at 95 °C, samples were subjected to 40 cycles, each consisting of 50 s at 95 °C, 60 s at 54 °C and 75 s at 72 °C; followed by a final extension step at 72 °C for 5 min. The amplification products were detected by agarose–gel electrophoresis, after staining with ethidium bromide.

The data were analysed using Yates corrected χ^2 test or Fisher's exact test, P values of less than 0·05 were taken as significant. Statistical analysis was performed using the Epi-Info 6 software (Centers for Disease Control and Prevention and World Health Organization).

Table 2. *Antimicrobial susceptibility of* S. aureus *isolates* ($n = 250$)

Agent	Resistance (%)
Penicillin	80·4
Methicillin	0·4
Gentamicin	13·2
Kanamycin	36·4
Tobramycin	14
Ciprofloxacin	3·2
Erythromycin	12·8
Clindamycin	7·6
Rifampin	18
Chloramphenicol	2·4
Tetracycline	2·8
Trimethoprim–sulphamethoxazole	0·4
Vancomycin	0
Teicoplanin	0

Two hundred and forty-eight subjects (30·5%) were identified as colonized with *S. aureus*. In total, 250 isolates were collected (two subjects were colonized with two different strains). The carriage rate for children and guardians was 35%, distinctively and on the overall (school community $n = 145$; children $n = 131$; guardians $n = 14$), and in the general population it was 25·9% ($n = 103$). The school community had a significantly higher colonization frequency than the general population ($P = 0{\cdot}006$) (Table 1). Subjects colonized with *S. aureus* were not distinguishable from non-carriers in terms of sex. There was no significant association of predisposing risk (available for the general population), considered individually and globally, between *S. aureus* carriers and non-carriers.

Antimicrobial resistance rates ranged from 0·4% for methicillin and trimethoprim–sulphamethoxazole to 80·4% for penicillin; no vancomycin or teicoplanin-resistant isolates were found (Table 2). Kanamycin resistance rates were found to be significantly higher in isolates from the school community ($n = 63$;

prevalence of 42·9%) than in isolates from the general population ($n = 28$; prevalence of 27·2%) ($P = 0{\cdot}02$; Yates corrected χ^2 test). No significant differences were found for the other antibiotics tested.

Only 1 of the 812 subjects sampled was identified as a carrier of MRSA (prevalence of 0·12%). The carrier was a 78-year-old man with predisposing risk factors (frequent hospitalization, history of recent surgery and antibiotic use). Presence of the *mecA* gene was detected in this isolate by means of PCR assay. The isolate was also resistant to aminoglycosides, ciprofloxacin, erythromycin, clindamycin, rifampin and tetracycline. Cultures obtained 4 months later, from the same subject, were MRSA-positive from nasal swab and negative from other sites (axilla and umbilicus). The isolate presented the same multi-resistant profile.

Antimicrobial susceptibility testing showed a 28·8% profile of multi-drug resistance ($\geqslant 3$ antibiotics). The most frequent multi-resistant pattern observed (18%; $n = 6$ in the general population and $n = 7$ in the school community) was the association of penicillin, gentamicin, kanamycin and tobramycin. Children and their guardians had a significantly higher colonization rate with multi-resistant *S. aureus* than the general population ($P = 0{\cdot}002$) (Table 1).

In this study, which to our knowledge represents the first study carried out in Italy, to detect nasal carriage of MRSA in the community, we isolated only one MRSA. The strain was from a subject with predisposing risk factors and showed a multi-resistant profile that suggested a nosocomial origin. The isolation of MRSA in our population, therefore, appeared to be a rare event, similar to what previously observed in the general adult population in Birmingham [11], and in a study population of children and their guardians in New York City [1]. On the basis of these data, some authors have warned of the need for caution before concluding that the community prevalence of MRSA is rising and ubiquitous, and our findings support this view [11]. In this study, in agreement with the literature, the prevalence of *S. aureus* nasal carrier in the community is 30·5% [12].

The finding of a significantly greater frequency of colonization of multi-resistant *S. aureus* in children and their guardians than of the general population can be interpreted in various ways: the scholastic community could be considered as a 'closed community' in which the spread of a microorganism (multi-resistant or not) could be facilitated, furthermore, it is known that children are more frequently *S. aureus* carriers [6]. Although data were only available for the general population, we did not find, in agreement with another report, a significant association between predisposing risk factors and the acquisition of *S. aureus* [1].

Regarding the antimicrobial susceptibility of methicillin-susceptible *S. aureus* (MSSA) our findings are similar to those reported for clinical isolates except for aminoglycosides and rifampin [13, 14]. In particular, as opposed to results from the SENTRY Antimicrobial Surveillance Program that indicated a rate of resistance to gentamicin lower than 5%, our data showed a percentage of resistance to aminoglycosides ranging from 13·2% (gentamicin) to 36·4% (kanamycin) [13].

Rifampin is a potent, bactericidal antistaphylococcal agent, but high-level resistant strains are easily selected *in vivo* if it is used alone [15]. Most MRSA isolates are resistant to multiple antibiotics and more than 50% are also resistant to rifampin, whereas 1% of MSSA isolates are resistant to rifampin [16]. Since a recent report suggests that rifampin could play a key role in the prevention of deaths caused by MRSA susceptible to rifampin with reduced susceptibility to vancomycin, glycopeptides are often combined with this drug for deep-seated staphylococcal infections [17]. In this view, the finding of 18% of MSSA resistant to rifampin in the studied population, should be of concern and emphasizes the need for a systematic surveillance for antibiotic resistance among *S. aureus* isolates circulating within communities.

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