

# Study of Methicillin-Resistant *Staphylococcus aureus* (MRSA) carriage in a population of HIV-negative migrants and HIV-infected patients attending an outpatient clinic in Rome

A. Oliva\*, M. Lichtner\*, M.T. Mascellino\*, M. Iannetta\*, A.M. Ialungo\*, S. Tadadjeu Mewamba\*, P. Pavone\*, F. Mengoni\*, C.M. Mastroianni\*, V. Vullo\*

*Key words: Methicillin-resistant Staphylococcus aureus (MRSA), HIV, migrants*

*Parole chiave: Staphylococcus aureus meticillino-resistente (MRSA), HIV, immigrati*

## Abstract

**Background:** Migration and HIV infection are known risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) carriage and infection. The aim of the study was to analyze the prevalence of MRSA nasal colonization in a high risk population of HIV-negative migrants and HIV-infected subjects. Secondary aim was to investigate over time MRSA carriage prevalence in HIV-infected subjects.

**Methods:** During the study period (January-June 2008), nasal swabs were collected from 96 HIV-negative migrants and 63 HIV-infected patients. A group of 68 seropositive subjects was additionally screened for MRSA carriage in 2012. Subjects were evaluated for HIV status, previous antibiotic use or hospitalization, soft tissue and skin infections (SSI), nationality and work conditions. The swab specimens were plated and incubated for 24-h under static condition at 37° and then identified as *S. aureus* by using standard methods.

**Results:** A total of 227 subjects, 131 HIV-infected adults (63 in 2008 and 68 in 2012) and 96 HIV-negative migrants, were analyzed. Overall, 71/227 (31.2%) were *S. aureus* carriers: 34 out of 131 (25.9%) among HIV infected subjects and 37 out of 96 (38.5%) among migrants. Two MRSA were detected in HIV-infected patients (2.8%). Between 2008 and 2012 there was an increase of MRSA carriage in HIV+ group ( $p=0.49$ ). No statistically significant differences were found between *S. aureus* carriers and no-carriers in terms of CD4+ cell count, TMP/SMX prophylaxis, previous antibiotic use or hospitalization, nationality and duration of stay in Italy. Among HIV+ patients there was a higher prevalence of SSI in MSSA carriers compared with no carriers (25% vs 4%,  $p=0.028$ ). In the migrants group, having a job based on a close human contact was significantly associated with *S. aureus* colonization ( $p=0.0038$ ).

**Conclusions:** Despite of the high prevalence of *S. aureus* isolation (31.2%), the present study showed the low rate of MRSA carriage in a high risk population. The main factor associated with *S. aureus* colonization was a close human contact rather than the HIV status and the condition of being migrant.

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\* Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy

## Introduction

Since its discovery in the second half of 1900s, methicillin-resistant *S. aureus* (MRSA) has been increasingly recognized as an important pathogen both in healthcare and community settings (1, 2). In recent years, community-acquired MRSA (CA-MRSA) has emerged as an threatening pathogen (3), causing outbreaks in athletes (4), military personnel (5), native Americans (6), injection drug users (IDUs) (7) and men who have sex with men (MSM) (8), thus highlighting that the spread of CA-MRSA is facilitated by a close human-to-human contact. *S. aureus* colonization is a risk factor for infection: patients colonized with MRSA have a two-to-twelve-fold increase of developing subsequent MRSA infection (9). HIV has been recognized as an independent risk factor for MRSA colonization and infection (10). On the other hand, migration has been showed to promote the diffusion and the circulation of different clones of MRSA (11-13).

In the present study, the prevalence and risk factors for MRSA colonization in a high risk population of HIV-infected subjects and migrants attending an ambulatory clinic in Rome was analyzed. Furthermore, it was investigated the temporal trend of MRSA carriage prevalence in HIV-infected subjects over time by comparing two different groups of subjects.

## Materials and methods

### *Study population*

A retrospective study among 159 subjects (63 adult HIV-infected subjects and 96 HIV seronegative migrants) attending the outpatients clinic of the Department of Infectious and Tropical Diseases of Sapienza University of Rome between

January and June 2008 was conducted. In addition, 68 seropositive subjects were screened for MRSA carriage in 2012. Thus, a total of 227 patients were included in the study.

Overall, subjects were evaluated for HIV status, previous antibiotic use or hospitalization and presence of soft tissue and skin infections (SSI) at the time of swabs collection. Antibiotic use and hospitalization were defined as antimicrobial assumption or hospital stay for any reason in the previous 6 and 12 months, respectively. SSI included impetigo, erysipela and cellulitis.

HIV staging, risk factors for HIV infection, antiretroviral (ARV) therapy and trimetoprim/sulfamethoxazole (TMP/SMX) prophylaxis were evaluated for all HIV-infected subjects. For migrants, additional informations including nationality, length of permanence in Italy and socio-economic status were collected. Furthermore, migrants were divided into two groups according to their working conditions: migrants having a job based on a close contact (baby-sitting, assistance to old people) and migrants having a job without a close contact (housewives, restaurant workers). Unemployed subjects were included in the second group. "Close contact" was defined as job contact which was considered to be intimate human-to-human contact. The study was approved by the institutional review board (Section of Infectious Diseases, Department of Public Health and Infectious Diseases, Sapienza University of Rome). All study participants gave informed written consent.

### *Sample collection*

Nasal swabs were obtained with a sterile polyester fiber-tipped swab that had been moistened with sterile saline (BBL™ Culture Swab™ Becton, Dickinson France). The swab specimens

obtained from the subjects' anterior nares were plated on Mannitol salt agar (Chapman medium, Oxoid). After a 24-h incubation under static condition at 37°C, positive colonies from each sample were spread onto 5% sheep blood agar plates (Becton, Dickinson France), and a single colony was selected for additional analysis. The golden haemolytic cultures were identified as *S. aureus* by means of the Staphyloslide agglutination test (Becton, Dickinson France) and by using the automated system VITEK-2 (Bio-Merieux, Marcy l'Etoile, France). Cultures positive for bacteria other than *S. aureus*, as coagulase negative staphylococci (CNS), were excluded from further analyses.

According to the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI) (14), oxacillin resistance in *S. aureus* was evaluated by using either oxacillin disks (6µg/ml) or the cefoxitin screening method (Oxoid) in Mueller-Hinton agar plates. The oxacillin-resistant strain were submitted to a full susceptibility test evaluation including gentamicin, erythromycin, glycopeptides, rifampin, TMP/SMX, fluoroquinolones, clindamycin and linezolid. Antimicrobial susceptibility testing (AST) was performed using the VITEK-2 system (Bio-Merieux, Marcy l'Etoile, France). All samples reached the microbiology laboratory within 3 hours from the collection.

#### Statistical analysis

Categorical variables were compared by using the  $X^2$  or Fisher's exact test, as appropriate. Continuous data were analyzed with Student's t-test whereas the non-parametric Mann-Whitney test was applied for values not normally distributed. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using STATA 9 software (STATA corp. LP, College Station, Texas, USA).

## Results

### *Characteristics of study population*

A total of 227 subjects were enrolled in the study, 131 HIV-infected adults (63 in 2008 and 68 in 2012) and 96 HIV-negative migrants. 93 were females, 134 were males, with a mean age of 38.7±10.6 years. Among HIV-infected subjects, 92 (70.2%) were males and the mean age was 46.2±9 years. The virological and immunological status were as follows: mean CD4+ cell count was 493±144.2 cell/mm<sup>3</sup>, mean viral load 130352 (range 0-500000) cp/ml. 129 patients (98.4%) were on antiretroviral therapy, 21 (16%) had prophylaxis with TMP/SMX. Considering the single risk factors for HIV infection, IDUs and MSM were 20 (15.2%) and 33 (25.2%), respectively. Six patients (4.5%) had SSIs at the time of swab collection. The mean age of migrants was 34±9.8 years (42 males, 54 females). The nationalities were the following: 37.5% from East Europe, 30.2% from Asian countries, 18.8% from Latin America and 13.5% from Africa. The mean length of stay in Italy was 4±2 years. 66 (69%) were employed, with 37 (38.5%) having a job based on a close human-to-human contact. Previous antibiotic therapy and/or hospitalization were present in 20 (15.2%) and 41 (42.7%) of HIV-positive patients and migrants, respectively.

### *S. aureus carriage*

Overall, microbiological analyses showed that 71/227 (31.2%) of the subjects were nasal *S. aureus* carriers: 34 out of 131 (25.9%) among HIV-infected subjects and 37 out of 96 (38.5%) among migrants. Among all *S. aureus* isolates, only 2 MRSA were detected (2.8%) (Figure 1).

All methicillin-susceptible *S. aureus* (MSSA) isolates were sensitive to linezolid, glycopeptides, rifampin, gentamicin,

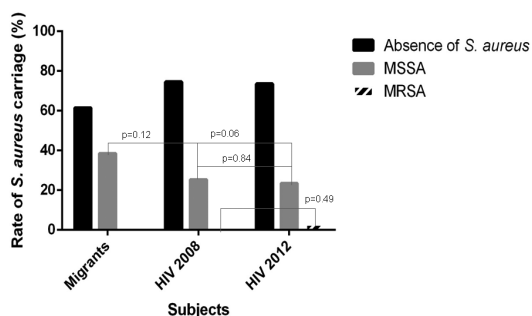


Figure 1 - *S. aureus* nasal colonization in the study population. During the study period, there was an increase of MRSA in HIV-infected subjects (0% vs 2.9%). Values are expressed as percentage.

erythromycin whereas 73.5% were resistant to penicillin, 3% to TMP/SMX, fluoroquinolones and tetracycline; 8.8% showed less sensitivity to clindamycin. One single strain resulted resistant to trimethoprim/sulfamethoxazole, fluoroquinolones, tetracycline and clindamycin. MRSA strains were sensitive to rifampin, linezolid, glycopeptides, resistant to trimethoprim/sulfamethoxazole, fluoroquinolones, erythromycin and clindamycin.

No statistically differences were found between MSSA carriers and no-carriers in terms of sex and age. Previous antibiotic

use or hospitalization did not significantly affect total carriage rates.

#### *HIV-infected subjects screened in 2008*

The rate of nasal *S. aureus* carriage was 25.4% (16/63). No MRSA was detected (Figure 1). The immunological and virological status, as well as TMP/SMX prophylaxis, did not significantly affect nasal carriage rates. Although the percentage of IDUs and MSM was higher in HIV+ carriers compared with HIV+ no-carriers (12% vs 9% and 37% vs 16%, respectively) this difference was not statistically significant ( $p = 0.63$ ;  $p = 0.16$ ). In contrast, there was a significant higher prevalence of SSI in MSSA carriers compared with no carriers (25% vs 4%,  $p = 0.028$ ) (Table 1).

#### *HIV-negative migrants*

*S. aureus* was detected in 37 out of 96 (38.5%) migrants. No MRSA grew (Figure 1). Among all the considered factors, there were no differences in terms of sex, age and median length of permanence in Italy between MSSA+ and MSSA- migrants. Interestingly, nationality did not significantly affect the colonization rate. 21 (57%) subjects who had a job based

Table 1 - *S. aureus* nasal colonization prevalence in HIV-infected subjects

	<i>S. aureus</i> carriers (N: 16)	<i>S. aureus</i> non-carriers (N: 47)	p-value
Age, yr (mean $\pm$ SD)	47 $\pm$ 9.7	46.6 $\pm$ 9.7	0.9
Males, no. (%)	11 (68.7%)	28 (59.5%)	0.56
Females, no. (%)	5 (31.3%)	19 (49.5%)	
Risk factor for HIV infection			
MSM, no. (%)	6 (37.5%)	8 (17%)	0.16
IDUs, no. (%)	2 (12.5%)	4 (8.5%)	0.63
Viro-immunological status			
CD4+, cell/mm <sup>3</sup> (mean $\pm$ SD)	468 $\pm$ 93	360 $\pm$ 64	0.2
HIV-RNA, cp/ml (mean $\pm$ SD)	5625 $\pm$ 2470	24989 $\pm$ 1900	0.3
SSI, no. (%)	4 (25%)	2 (4%)	0.028
TMP/SMX prophylaxis, no. (%)	4 (25%)	15 (31%)	0.18

Table 2 - *S. aureus* nasal colonization prevalence in HIV-negative migrants.

	<i>S. aureus</i> carriers (N: 37)	<i>S. aureus</i> non-carriers (N: 59)	p-value
Age, yr, no. (%)			0.18
< 30 yr	16 (43.2%)	17 (28.8%)	
30-49yr	10 (27%)	21 (35.6%)	
> 49yr	11 (29.8%)	21 (35.6%)	
Males, no. (%)	16 (43.2%)	26 (44.1%)	0.56
Females, no. (%)	21 (56.8%)	33 (55.9%)	
Length of stay in Italy, no. (%)			0.52
≤ 5 years	28 (75.7%)	48 (81.4%)	
> 5 years	9 (24.3%)	11 (18.6%)	
Nationality, no. (%)			0.55
Africa	6 (15.8%)	7 (11.8%)	
Asia	11 (30%)	17 (28.9%)	
East-Europe	14 (38%)	23 (39%)	
Latin America	6 (16.2%)	12 (20.3%)	
Living conditions, no. (%)			0.06
same nationality/family	26 (70.3%)	51 (86.4%)	
with employer	11 (29.7%)	8 (13.6%)	
Working conditions, no. (%)*			0.0038
Job with close contact	21 (61.8%)	16 (29.1%)	
Job without close contact	13 (38.2%)	39 (70.9%)	

\* Missing data due to linguistic difficulties with migrants.

on a close contact were more frequently MSSA carriers, in comparison to 16 (27.1%) subjects who did not have a job with human contact ( $p = 0.0038$ ). Migrants living with the employer showed a higher rate of *S. aureus* carriage when compared with those living with their family or subjects with the same nationality (57.9% vs 33.8%, respectively;  $p = 0.06$ ) (Table 2).

#### *HIV-infected subjects screened in 2012.*

A total of 68 HIV-infected subject was screened for MRSA nasal carriage in 2012. The demographic characteristics and the presence of risk factors for HIV infection were similar to those of the previous group of HIV-infected patients. Mean CD4+ cell count was  $595 \pm 131$  cell/mmc whereas the HIV-RNA was detectable in only 3/68 (4.4%) subjects. The

majority of patients (67/68, 98.5%) were on antiretroviral therapy, 2 (2.9%) had prophylaxis with TMP/SMX. Previous antibiotic therapy and/or hospitalization were present in 3 (4.4%) of the patients. Overall, 16/68 were MSSA carriers (23.5%). In contrast with the lack of MRSA carriage previously observed, 2 out of 68 (2.97%) were positive for MRSA. However, this increase was not statistically significant ( $p = 0.49$ ) (Figure 1). No association between HIV staging, risk factors, presence of SSI at the moment of sample collection and MRSA colonization was found.

## Discussion

MRSA infections are a major threat worldwide (15). *S. aureus* colonization is

a predominant risk factor for subsequent infection: MRSA infection is 4 times more frequent in MRSA carriers than in noncarriers (16). The principal reservoir of *S. aureus* is the nasopharynx (17), although several studies identified throat, arm pit, perineum and gastrointestinal tract as other possible sites of *S. aureus* carriage (18, 19). The occurrence of CA-MRSA outbreaks in athletes, military personnel, IDUs, MSM and prisoners supports the hypothesis that the acquisition and the spread of this strain need a close human-to-human contact.

Despite a high rate of infections due to MRSA (20), Italy is one of the European countries where the prevalence of MRSA carriage is low (21, 22). Current guidelines for MRSA prevention and control (23) highlight the importance of controlling MRSA spread by early recognition of colonized patients, especially in high risk populations.

HIV-positive subjects have a higher rate of *S. aureus* colonization than general population (24). The strong association between HIV infection and *S. aureus* colonization could explain the greater incidence of infections due to MRSA in this population (25). Despite the reasons for the association between HIV infection and *S. aureus* remain unknown, environmental factors and the immunologic HIV-related abnormalities could play a pathogenetic role (25). Behavioral risk factors (i.e. MSM, IDUs) and the immunological status with a low CD4+ cell count have been associated with MRSA colonization (25). In contrast, the use of TMP/SMX as a prophylaxis appeared to be protective (24). Our study confirmed the high rate of *S. aureus* nasal carriage in HIV-infected subjects (25.9%). A **significant association** between MSSA carriers and the presence of SSIs at the moment of sample collection was found, thus suggesting the importance of skin-to-skin contact in

determining both colonization and infection. In contrast with other studies (24, 25), **immunological status did not correlate** with colonization. Instead, a study conducted by Miller *et al* (26) showed that CD4+ cell count was not associated with prevalent, incident or persistent *S. aureus* carriage. Overall, we found a low prevalence of MRSA nasal colonization. However, when the study population was stratified according to the different years of sample collection, we showed that the detection of MRSA rose up to 2.9% in 2012 compared with the lack of MRSA detection in 2008. Although not statistically significant ( $p=0.49$ ), this finding highlights that HIV-infected subjects are at risk of MRSA nasal colonization.

Migration is another known risk factor for MRSA carriage. In fact, in countries with a low MRSA prevalence, transmission of imported cases of MRSA is one of the crucial risk factors that influence the risk for MRSA acquisition (27). Studies conducted in Sweden and Norway among foreign children demonstrated an intra-familial transmission of methicillin-resistant clones from adopted children coming from countries where MRSA was highly prevalent (12, 13). Furthermore, a Spanish study showed that among CA-MRSA carriers, most of them were immigrants (28) and a Danish study highlighted that non-Danish origin was the only independent risk factor associated with CA-MRSA infections (29). These results strongly suggest that the increase of the migratory flows in the world could contribute to the spread of MRSA, even in countries where the prevalence of MRSA is still low. In the present study, despite the absence of MRSA detection, we found a high rate of *S. aureus* nasal carriage among migrants (38.5%). The main factor associated with *S. aureus* colonization was close human-to-human contact (i.e. work conditions). These findings highlight how important

is the human contact for the spread of *S. aureus* strains. Moreover, we found that migrants who lived with the employers were *S. aureus* carriers more frequently than migrants who lived together or with their family, thus suggesting that not only migrants could favour the spread of *S. aureus* clones, but also living with Italian people could expose migrants to the risk of being colonized.

In conclusion, despite the high prevalence of *S. aureus* isolation (31.2%), the study showed a low detection rate of MRSA nasal carriage in a high risk population of HIV-infected subjects and migrants. The main factor associated with *S. aureus* colonization was a close human-to-human contact rather than HIV status and the condition of being migrant.

## Riassunto

**Studio della colonizzazione nasale da *Staphylococcus aureus* meticillino-resistente (MRSA) in una popolazione di immigrati HIV-negativi e soggetti HIV-positivi in un ambulatorio di Roma**

**Background:** La migrazione e l'infezione da HIV sono fattori di rischio sia per la colonizzazione che per l'infezione da *Staphylococcus aureus* meticillino-resistente (MRSA). Scopo dello studio è stato quello di analizzare la prevalenza della colonizzazione nasale da MRSA in una popolazione ad elevato rischio di pazienti HIV-positivi e immigrati HIV-negativi. Inoltre, è stata analizzata nel tempo la prevalenza della colonizzazione da MRSA nei soggetti HIV-positivi.

**Metodi:** Durante il periodo dello studio (Gennaio-Giugno 2008), sono stati effettuati tamponi nasali in 96 immigrati HIV-negativi e 63 soggetti HIV-positivi. Un addizionale gruppo di 68 pazienti con infezione da HIV è stato analizzato per colonizzazione da MRSA nel 2012. Per ciascun soggetto sono stati considerati: la presenza di infezione da HIV, una precedente terapia antibiotica o un precedente ricovero, la presenza di infezione della cute e dei tessuti molli al momento della raccolta del campione, la nazionalità e le condizioni di lavoro. I campioni raccolti sono stati incubati a 37° per 24h e i microorganismi identificati secondo le tecniche microbiologiche standard.

**Risultati:** Un totale di 227 pazienti, 131 adulti con infezione da HIV (63 nel 2008 e 68 nel 2012) e 96

immigrati HIV-negativi sono stati inclusi nello studio. Globalmente, 71/227 (31.2%) erano colonizzati da *S. aureus*: 34/131 (25.9%) tra gli HIV-positivi, 37/96 (38.5%) tra gli immigrati. Solo 2 MRSA sono stati identificati, nel gruppo dei soggetti con infezione da HIV. Nel periodo tra il 2008 e il 2012, è stato notato un incremento della colonizzazione nasale da MRSA all'interno dei pazienti HIV-positivi: dallo 0% del 2008 al 2.8% del 2012 ( $p=0.49$ ). Tra i soggetti colonizzati e quelli non colonizzati, non sono state trovate differenze statisticamente significative in termini di *status* viro-immunologico, precedenti ricoveri o terapie antibiotiche, nazionalità e, per gli stranieri, durata della permanenza in Italia. Tra gli HIV-positivi, è stata trovata una maggiore prevalenza di infezioni della cute e dei tessuti molli nei colonizzati rispetto ai non colonizzati (25% vs 4%,  $p=0.028$ ). Nel gruppo degli immigrati, avere un lavoro che presupponeva uno stretto contatto umano risultava statisticamente associato alla colonizzazione da *S. aureus* ( $p=0.0038$ ).

**Conclusioni:** Nonostante una elevata prevalenza di isolamento di *S. aureus* (31.2%), il presente studio dimostra il basso tasso di colonizzazione da MRSA in una popolazione considerata ad alto rischio. Il principale fattore associato alla colonizzazione da *S. aureus* è risultato essere il contatto interumano piuttosto che la presenza dell'infezione da HIV o la condizione di migrante.

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Corresponding author: Prof. Vincenzo Vullo, Department of Public Health and Infectious Diseases, Sapienza University, Viale del Policlinico 155, 00161 Rome, Italy  
e-mail: vincenzo.vullo@uniroma1.it

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