Methicillin-resistant Staphylococcus aureus nasal carriage among healthy employees of the Hellenic Air Force

S Karapsias[1], E T Piperaki (epiper@med.uoa.gr)[2], I Spiliopoulou[3], G Katsanis[1], A Tseleni - Kotsovili[2]
1. Department of Microbiology, Air Force General Hospital, Athens, Greece
2. Department of Microbiology, School of Medicine, University of Athens, Greece
3. Department of Microbiology, School of Medicine, University of Patras, Greece

The prevalence of methicillin-resistant Staphylococcus aureus nasal carriage among 959 healthy employees of the Hellenic Air Force was investigated from November 2004 to October 2005. Nine participants were found to be colonised by methicillin-resistant Staphylococcus aureus (MRSA) (SCCmec type IV). Eight of the MRSA isolates were PVL-negative and belonged to ST30 by MLST, while the remaining one isolate was PVL-positive and classified as ST-80.

Introduction

The incidence of infections caused by methicillin-resistant Staphylococcus aureus (MRSA) apparently acquired in the community (CA-MRSA) is increasing. CA-MRSA isolates are commonly non-multi-drug resistant and belong to lineages distinct from those of MRSA strains prevailing in hospitals [1]. Recent reports from Greece indicated community emergence of MRSA mainly implicated in skin and soft tissue infections in children [2,3]. Yet, the extent of the spread of CA-MRSA in the community has not been studied. We attempted to evaluate the prevalence as well as the microbiological and epidemiological characteristics of MRSA strains in a population of healthy adults in Greece.

Methodology

The study population consisted of employees of the Hellenic Air Force (HAF), residing in different geographical areas of Greece, visiting the Air Force General Hospital in Athens from November 2004 to October 2005, for a scheduled biannual medical examination. Before joining the HAF, all participants had been in good health. For operational reasons, they trained and maintained good physical fitness. Additionally, they underwent an obligatory medical examination at least once every two years. Therefore, this study population was considered as approximating "healthy adults". Demographic data and medical history over the preceding year, including hospitalisation, surgery, use of antibiotics or other medication and underlying diseases, were obtained for each participant during a short interview by a medical doctor.

Swabs obtained from both anterior nares of each individual were immediately streaked onto mannitol salt agar containing 2 µg/ml oxacillin (Oxacillin Resistance Screening Agar Base, Oxoid Ltd.). Plates were incubated at 35ºC for 48 h. Colonies demonstrating an intense blue colour were subcultured onto blood agar and incubated overnight at 35ºC. Species identification was performed by standard methods. Susceptibility profile to a wide variety of antimicrobial agents was determined by the disk diffusion method according to the current CLSI guidelines. Isolates were also tested by an oxacillin disk (1 µg) and a cefoxitin disk (30 µg) to confirm methicillin resistance. MRSA isolates were defined as community-associated according to established criteria [4].

MRSA isolates were characterised by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) of chromosomal DNA SmaI digests. Macrorestriction patterns were compared to previously identified clones [5]. Multi-locus sequence typing (MLST) was performed to all PFGE/SCCmec types. MRSA were additionally characterised by spa typing. Sequences of amplified parts of the spa gene were analysed using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany). Detection of mecA as well as SCCmec typing was carried out by PCR. Genes lukS-PV and lukF-PV encoding Panton-Valentine leukocidin (PVL) were also identified.

Data were processed and analysed by using the SPSS statistics software, version 12 for Windows. Bivariable comparisons were carried out by the $\chi 2$ or Fisher's exact test for categorical variables and the t-test for continuous variables.

Results

A total of 959 individuals (874 males) aged 18 to 60 years (mean age 33) were enrolled in the study. Nine of the 959 participants (0.94%, 95% confidence interval [CI] 0.33% to 1.55%) were colonised with MRSA. All MRSA carriers were males. Two of the colonised individuals were smokers. One of the MRSA carriers reported systematic use of inhaled corticosteroids during the two months preceding enrolment. Another carrier had been treated with antibiotics two months prior to sampling. Three of the colonised individuals had been admitted to different hospitals at least once in the year before enrolment in the study. Two of them had been hospitalised in medical wards while the third one had been admitted to a surgical ward. In two MRSA carriers none of the investigated risk factors was identified. Among the demographic and clinical variables, prior hospitalisation and use of inhaled corticosteroids appeared to be correlated with an increased risk for MRSA colonisation ($P<0.01$) (Table 1).

Characteristics of the MRSA isolates are presented in Table 2. All nine isolates were susceptible to imipenem, gentamycin, erythromycin, clindamycin, ciprofloxacin, trimethoprim-

sulfamethoxazole, rifampicin, linezolid, teicoplanin and vancomycin. One isolate (Sa-344) was resistant to tetracycline (Tet) and two isolates (Sa-344, Sa-784) exhibited intermediate susceptibility to fusidic acid (Fus).

Eight isolates exhibited similar PFGE patterns (type A) not differing by more than three bands, correlated to ST30 by MLST. The chromosomal fingerprint of isolate Sa-344 was distinct (type C) belonging to ST80 by MLST. A total of five spa types were identified. Five of the eight ST30 isolates were classified as t012 (three strains) and t018 (two strains) that are common among strains of this ST. ST80 strain was classified as t044, a spa type strongly associated with this particular lineage. SCCmec typing revealed that all isolates possessed the SCCmec type IV. Genes lukF-PV and lukS-PV encoding PVL were detected only in the ST80 isolate.

Discussion

This study confirms the circulation of PVL-positive t044/ST80-IV which is common among CA-MRSA in Europe [6] as well as several spa variants of a PVL-negative ST30-IV MRSA frequently encountered in Greek hospitals [5]. While only one of the nine isolates belonged to ST80, this type seems to predominate among community-acquired infections requiring hospitalisation [2,3] most likely reflecting a higher virulence. In addition, since the PVL-positive strain was one of the two fusidic acid-resistant MRSA

Table 1

Risk factors tested for MRSA colonisation, study of Hellenic Air Force employees, Greece, 2004-2005 (n=959)

Characteristics	Number (%) of MRSA-colonised subjects	Total number of subjects	Statistically significant difference
Sex			P>0.05
Male	9 (1.03)	874	
Female	0 (0)	85	
Smoking			P>0.05
No	7 (1.39)	501	
Yes	2 (0.44)	458	
Antibiotic use (within the past two months)			P>0.05
No	8 (0.89)	902	
Yes	1 (1.75)	57	
Corticosteroid use (within the past two months)			
No	8 (0.85)	943	
Yes (inhaled)	1 (10)	10	P<0.01
Yes (*per os*)	0 (0)	6	
Hospitalisation (during the past year)			
No	6 (0.71)	844	
Yes (medical ward patients)	2 (6.25)	32	P<0.01
Yes (surgical ward patients)	1 (1.2)	83	

Table 2

Characteristics of nine CA-MRSA isolates from healthy carriers, study of Hellenic Air Force employees, Greece, 2004-2005

Isolate	Resistance to non-β-lactams	PFGE type (MLST)	mecA type	spa type	PVL	Factors potentially associated with MRSA colonisation
43	-	A (ST30)	IV	t1051	-	Smoking
196	-	A (ST30)	IV	t046	-	Antibiotics
344	Tet, Fus	C (ST80)	IV	t044	+	Hospitalisation (medical ward)*
408	-	A (ST30)	IV	t046	-	Inhaled corticosteroids*
714	-	A (ST30)	IV	t018	-	-
778	-	A (ST30)	IV	t018	-	-
784	Fus	A (ST30)	IV	t012	-	Hospitalisation (surgical ward)
901	-	A (ST30)	IV	t012	-	Smoking
933	-	A (ST30)	IV	t012	-	Hospitalisation (medical ward)*

* Denotes factors that appeared as significantly associated with MRSA colonisation

isolates, the emergence of MRSA with fusidic acid resistance could be a convenient means for the timely detection of any increase in the incidence of PVL-positive MRSA in the community [7].

Differences in MRSA colonisation rates of apparently healthy community-dwelling persons have been observed in various settings. In western European countries colonisation rates are comparable to the rate observed here in Greece [6]. In other countries such as Taiwan, however, the respective rate is as high as 3.5% and has been partly attributed to the excessive community use of antibiotics [8]. Although consumption of antibiotics in Greece ranks among the highest in Europe, the MRSA isolation rate in this study was relatively low. This could be partly due to the fact that the study population was composed of individuals healthier than average adults and with limited exposure to antibiotics and healthcare.

Eight of the isolates were indistinguishable from the ST30 strain that has been established in Greek hospitals [5,9]. Notably, three of the eight respective carriers had been admitted to a hospital at least once in the year preceding enrolment in the study. Hence, a hospital origin of the ST30 strains circulating in this community cannot be excluded.

References

1. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, et al. The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect. 2007;13(3):222-35.
2. Vourli S, Perimeni D, Makri A, Polemis M, Voyiatzi A, Vatopoulos A. Community acquired MRSA infections in a paediatric population in Greece. Euro Surveill. 2005;10(5):pii=537. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=537.
3. Chini V, Petinaki E, Meugnier H, Foka A, Bes M, Etienne J, et al. Emergence of a new clone carrying Panton-Valentine leukocidin genes and staphylococcal cassette chromosome mec type V among methicillin-resistant Staphylococcus aureus in Greece. Scand J Infect Dis. 2008;40(5):368-72.
4. Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG. Community-associated methicillin-resistant Staphylococcus aureus isolates causing healthcare-associated infections. Emerg Infect Dis 2007;13(2):236-42.
5. Aires de Sousa M, Bartzavali C, Spiliopoulou I, Sanches IS, Crisóstomo MI, de Lencastre H. Two international methicillin-resistant Staphylococcus aureus clones endemic in a university hospital in Patras, Greece. J Clin Microbiol. 2003;41(5):2027-32.
6. Tiemersma EW, Bronzwaer SL, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, et al. Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002. Emerg Infect Dis. 2004;10(9):1627-34.
7. Witte W, Cuny C, Strommenger B, Braulke C, Heuck D. Emergence of a new community acquired MRSA strain in Germany. Euro Surveill. 2004;9(1):pii=440. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=440
8. Lu PL, Chin LC, Peng CF, Chiang YH, Chen TP, Ma L, et al. Risk factors and molecular analysis of community methicillin-resistant Staphylococcus aureus carriage. J Clin Microbiol. 2005;43(1):132-9.
9. Chini V, Petinaki E, Foka A, Paratiras S, Dimitracopoulos G, Spiliopoulou I. Spread of Staphylococcus aureus clinical isolates carrying Panton-Valentine leukocidin genes during a 3-year period in Greece. Clin Microbiol Infect 2006;12(1):29-34.

This article was published on 2 October 2008.

Citation style for this article: Karapsias S, Piperaki ET, Spiliopoulou I, Katsanis G, Tseleni - Kotsovili A. Methicillin-resistant Staphylococcus aureus nasal carriage among healthy employees of the Hellenic Air Force . Euro Surveill. 2008;13(40):pii=18999. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18999