Journal of Antimicrobial Chemotherapy (2004) **53**, 105–108
DOI: 10.1093/jac/dkh028
Advance Access publication 4 December 2003

JAC

Clonal dissemination of mupirocin-resistant staphylococci in Greek hospitals

E. Petinaki[1], I. Spiliopoulou[2], F. Kontos[1]†, M. Maniati[1]†, Z. Bersos[1], N. Stakias[1], H. Malamou-Lada[3], Ch. Koutsia-Carouzou[4] and A. N. Maniatis[1]*

[1]Department of Microbiology, School of Medicine, University of Thessalia, Papakyriazi 22, Larissa; [2]Department of Microbiology, School of Medicine, University of Patras; [3]Department of Microbiology, General Hospital of Athens G. Genimatas, Athens; [4]Department of Microbiology, General Hospital Asclepeion, Voula, Athens, Greece

Received 23 July 2003; returned 1 September 2003; revised 23 September 2003; accepted 10 October 2003

***Objectives*: To determine the rates of mupirocin resistance in staphylococci during a 4 year period (1999–2002) in Greece.**

***Materials*: A total of 1200 *Staphylococcus aureus* and 2760 coagulase-negative staphylococci (CoNS), consecutively collected from four Greek hospitals located in different geographical areas, were tested for susceptibility to mupirocin using the Etest and a reference agar dilution method.**

***Results*: Twenty-four *S. aureus* (2%) and 532 CoNS (19.2%) were found to be mupirocin-resistant during the study period. High-level mupirocin resistance was detected in 20 *S. aureus* (1.6%) and in 440 CoNS (15.9%), respectively. No variations in the rates of mupirocin-resistant *S. aureus* in relation to the year of collection were observed. In contrast, the rate of mupirocin-resistant CoNS increased dramatically from 9% in 1999, to 14% in 2000, 20% in 2001 and reached 33% in 2002. PFGE analysis revealed the presence of one main clone (A) among mupirocin-resistant *S. aureus* and two main clones (i and a) among *Staphylococcus epidermidis* isolates.**

***Conclusions*: In Greece, the rate of mupirocin-resistant *S. aureus* has remained low and steady since 1999. The high rate of mupirocin-resistant CoNS (33%) in 2002 was due mainly to clonal dissemination of epidemic hospital clones.**

Keywords: *Staphylococcus aureus*, *Staphylococcus epidermidis*, mupirocin resistance, epidemic clones, Greece

Introduction

The elimination of staphylococci, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), from the nose plays a crucial role in infection control protocols. Currently, one of the most effective topical agents for eradication of nasal carriage of MRSA is mupirocin.[1] This antimicrobial agent is also used to prevent catheter colonization by coagulase-negative staphylococci (CoNS). However, staphylococcal isolates resistant to mupirocin are found worldwide.[2,3] Staphylococci expressing mupirocin resistance can be divided in two groups: low-level resistant (MuL) with MICs in the range 8–256 mg/L and high-level resistant (MuH) with MICs ≥ 512 mg/L. Low-level resistance to mupirocin is more common and is thought to arise from point mutations within the usual chromosomal staphylococcal isoleucyl-tRNA synthetase gene (*ileS*).[1] High-level resistance results from acquisition of a transferable plasmid carrying a new gene, *ileS-2*, encoding a second novel staphylococcal isoleucyl-tRNA synthetase, which has no affinity to mupirocin.[1] Low and high-level resistance has been detected in both *S. aureus* and CoNS.

In Greece, mupirocin is only used to eradicate nasal carriage of MRSA in patients and staff. The antibiotic is not used for the treatment of staphylococcal skin infections or for the prevention of bacterial colonization due to coagulase-negative staphylococci. In the present study, we investigated the rate of development of mupirocin-resistant staphylococci (*S. aureus* and CoNS) in Greek hospitals during 1999–2002.

Materials and methods

Bacterial isolates

A total of 3960 staphylococci—comprising 1200 *S. aureus* and 2760 CoNS, consecutively isolated during January 1999–December 2002,

†These authors contributed equally to this work.
*Corresponding author. Fax: +30-41-0682508; E-mail: anm@otenet.gr

Table 1. Distribution of low- and high-level mupirocin resistance among staphylococci in correlation to the time of isolation

	Year	Number of isolates	MuL		MuH		
			isolates	%	isolates	%	% Mu
S. aureus							
	1999	240	2	0.8	4	1.66	2.5
	2000	360	0		6	1.66	1.6
	2001	280	2	0.7	4	1.4	2.14
	2002	320	0		6	1.8	1.8
	total	1200	4	0.33	20	1.6	2
Coagulase-negative staphylococci							
	1999	660	0		60	9	9
	2000	700	22	3.14	76	10.8	13.99
	2001	680	34	5	102	15	20
	2002	720	36	5	202	28	33
	total	2760	92	3.3	440	15.94	19.24

MuL, low-level mupirocin resistance; MuH, high-level mupirocin resistance; Mu, mupirocin resistance.

associated with blood, skin and soft tissue infections, and recovered from clinically significant specimens—were included in the study. The samples were collected from four tertiary care Greek hospitals, located in three geographical areas (Athens, Central Greece and Southwestern Greece). Isolates recovered from different cultures (blood, catheter etc.) from the same patient with the same *Sma*I pulsotype and the same antibiotic resistance profile were included once. Identification at the species level was carried out by Gram stain, catalase and coagulase tests, and by the API Staph System (bioMerieux, SA Lyon, France).

Susceptibility tests

All isolates were tested using the mupirocin Etest (AB BIODISK, Solna, Sweden), and interpretation of susceptibility test results was conducted following the recommendations of the mupirocin manufacturer. Susceptibility results obtained by Etest were compared with those obtained after MIC determination using the reference agar dilution method.[4] Potential co-resistance to 14 antimicrobial agents (ampicillin, oxacillin, trimethoprim/sulfamethoxazole, ofloxacin, clindamycin, erythromycin, gentamicin, tobramycin, rifampicin, tetracycline, fusidic acid, vancomycin, linezolid and quinupristin/dalfopristin) was also determined by the agar diffusion method.[5]

Detection of ileS-2 and mecA genes

All isolates were tested for the presence of *ileS-2* and *mecA* genes by PCR, as described previously.[6] The predicted size of the PCR products were 456 bp and 310 bp for the *ileS-2* and *mecA* fragments, respectively.

PFGE analysis

Molecular typing of the mupirocin-resistant isolates was performed by PFGE analysis.[7,8] The banding patterns of the strains were compared visually following the criteria of Tenover *et al.*[7]

Results

A total of 556 staphylococci were found to be mupirocin-resistant by both agar dilution and Etest (MIC ≥ 8 mg/L). No discrepancies were observed between the reference agar dilution and Etest MIC values. The MuL staphylococcal strains with mupirocin MICs in the range 8–256 mg/L were easily recognized by the Etest, having a faint but visible zone of inhibition around the Etest strips. The MuH staphylococcal strains with mupirocin MICs ≥ 512 mg/L all had heavy, confluent growth with no detectable zones around the Etest strips.

Among the 1200 *S. aureus* isolates, 24 (2%) expressed mupirocin resistance during the study period. These 24 isolates were collected from patients; none of them had taken mupirocin treatment for nasal carriage. The distribution of low- and high-level mupirocin resistance in relation to time of isolation is described in Table 1. The rate of mupirocin resistance among *S. aureus* isolates was low and has remained steady since 1999. MuL was detected only in four *mecA*-positive *S. aureus* isolates (MIC 32 mg/L), belonging to clones A (three) and B (one), which have spread in several Greek hospitals.[8] MuH (MIC ≥ 512 mg/L) was detected in 20 *S. aureus* isolates (14 *mecA*-positive), sporadically isolated in two of the four participating hospitals. PFGE analysis revealed that all of the MuH strains belonged to clone A, which expressed a relatively susceptible phenotype (Table 2).

Among the 2760 CoNS isolates, 1932 were identified as *Staphylococcus epidermidis*, 400 as *Staphylococcus haemolyticus*, 380 as *Staphylococcus hominis*, 14 as *Staphylococcus saprophyticus*, 14 as *Staphylococcus simulans*, 10 as *Staphylococcus lugdunensis* and 10 as *Staphylococcus xylosus*. Mupirocin resistance was detected in 532 clinically significant isolates, comprising 528 *S. epidermidis*, one *S. haemolyticus*, one *S. hominis*, one *S. lugdunensis*, and one *S. xylosus*. The respective infections were distributed evenly over the study period and there was no evidence of outbreaks. Among mupirocin-resistant CoNS, only 10 *S. epidermidis* isolates (four expressing MuL and six expressing MuH) were collected from patients after mupirocin treatment for nasal carriage. The distribution of MuL and MuH in relation to the time of isolation is described in Table 1. MuL (MIC 8–64 mg/L) was detected in 92 *S. epidermidis* isolates, of which 86 isolates were *mecA*-positive. MuH (MIC ≥ 512 mg/L) was detected in 440 isolates (436 *S. epidermidis*, one *S. haemolyticus*, one

Table 2. Genotypic and phenotypic properties of mupirocin-resistant *S. aureus* and *S. epidermidis*

	Number of isolates	MuL	MuH	PFGE type	Antibiotic resistance
S. aureus					
	23		6	A	AMP
		3	14	A	AMP, OXA
	1	1		B	AMP, OXA, ERY, CIP, FUS, SXT
S. epidermidis					
	292	6	286	i	AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT,OFX
	64	64	–	a	AMP, OXA,ERY, CLI, FUS, TOB, SXT, OFX, RIF
	62	6	56	d	AMP, OXA, ERY, FUS, GEN
	44	2	42	b	AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT, OFX
	10	6	4	c	AMP, TET, FUS
	8	8	–	e	AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT, OFX, RIF
	24	–	24	g	AMP, OXA, ERY, CLI, FUS, TET, TOB
	24	–	24	l	AMP, OXA, ERY, FUS, TET, TOB

MuL, low-level mupirocin resistance; MuH, high-level mupirocin resistance; AMK, amikacin; AMP, ampicillin; CLI, clindamycin; OFX, ofloxacin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; TOB, tobramycin; OXA, oxacillin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

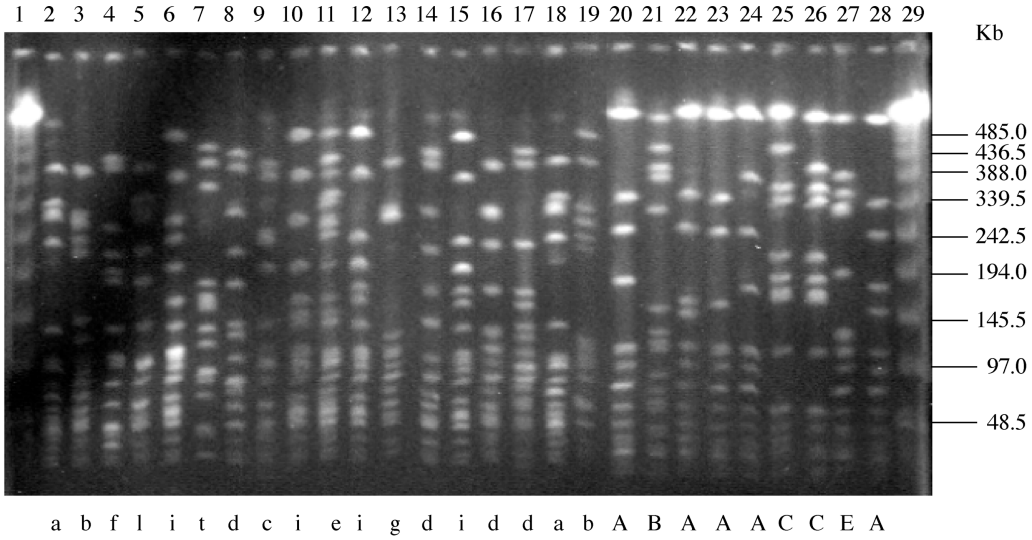

Figure 1. PFGE of *Sma*I macrorestriction fragments of mupirocin-resistant *S. epidermidis* and *S. aureus* clinical isolates. Lanes 1 and 29, molecular size standards (lambda oligomers); numbers at right show molecular sizes in kilobases; small letters in the bottom indicate PFGE types of *S. epidermidis* and capital letters PFGE types of *S. aureus.* Lanes 2–7: representatives of epidemic methicillin-resistant *S. epidermidis* clones previously characterized;[9] lanes 8–19: mupirocin-resistant *S. epidermidis* strains; lanes 20–24: mupirocin-resistant *S. aureus* strains; lanes 25–28: representatives of epidemic MRSA clones previously characterized.[8]

S. hominis, one *S. lugdunensis*, and one *S. xylosus*), of which 436 were *mecA*-positive. The rate of mupirocin resistance increased dramatically from 9% in 1999, to 14% in 2000, 20% in 2001 and reached 33% in 2002. Significant differences in the rates of resistance among hospitals have not been observed, although these hospitals belong to totally different geographic (rural, urban) areas. Furthermore, no correlation was found between the site of infection and the mupirocin-resistance rate. CoNS exhibited resistance to more than four classes of antimicrobial agents (Table 2).

As expected, all MuH isolates carried the *ileS-2* gene, which was not detected in any MuL isolate. Analysis by PFGE showed that, although MuH *S. epidermidis* strains fell into six distinct clones (i, d, b, g, l, c), the great majority of isolates, 286 out 436 (65.6%), belonged to clone i (Figure 1).[9] Before 1999, strains belonging to this clone did not carry the *ileS-2* gene, so the resistant mutants have emerged in the last 4 years (data not shown). The MuL *S. epidermidis* strains were grouped into six different clones (a, e, i, d, c, b), the most dominant being clone a, comprising 64 out of 92 strains (69.56%).

PFGE types a, b, i and l have been characterized previously as epidemic clones.[9] PFGE types d, e, c and g emerged later, after 2000.

Discussion

During the last decade, the increasing number of methicillin-resistant *S. aureus* worldwide has resulted in greater use of topical application mupirocin to prevent colonization and subsequent infection. However, the use of mupirocin, especially after prolonged duration of topical treatment and/or in areas of highly concentrated drug, such as skin infections and burns, leads to the emergence of resistance.

Mupirocin resistance is relatively unusual in *S. aureus,* but it is common and increasing in CoNS. It varies greatly from institution to institution regardless of geographic region monitored. According to the SENTRY antimicrobial surveillance programme 2000, mupirocin resistance rates from bloodstream infections varied by geographic area (USA, Canada, Latin America and Europe) for *S. aureus* from 1.9% to 5.6% and for CoNS from 12.8% to 39.9%.[2] A previous study in 19 European hospitals in 12 countries reported high-level resistance in 1.6% of *S. aureus* and 5.6% of CoNS isolates, and low-level resistance in 2.3% of *S. aureus* and 7.2% of CoNS isolates.[3]

The prevalence of mupirocin-resistant *S. aureus* in Greek hospitals in this study is lower than that reported in a previous study (1.8% in 2002 versus 4.5% in 1997).[10] However, the rate of mupirocin-resistant CoNS has increased dramatically, ranging from 9% in 1999 to 33% in 2002. The predominance of the clones A (among MuH *S. aureus*, which has spread in several Greek hospitals),[8] and i and a (among MuH and MuL *S. epidermidis* strains), already characterized as epidemic clones,[9] suggests that a limited number of mupirocin-resistant clones has been disseminated in the Greek hospital environment. This is not surprising for chromosomally mediated MuL, but is less expected for plasmid-mediated MuH, where horizontal spread of the plasmid among genetically diverse strains is likely. The high prevalence of mupirocin-resistant staphylococci was due mainly to clonal dissemination and to a lesser extent to gene spread.

The resistance profiles of the isolates have shown that the overwhelming majority of these were resistant to methicillin. Linezolid, quinupristin/dalfopristin and vancomycin maintained high activity against essentially all mupirocin-resistant strains.

In the period 1999–2002 in Greece, a rising incidence of mupirocin-resistant CoNS has been observed. In contrast, mupirocin resistance in *S. aureus* has remained more constant. In our hospitals, the use of mupirocin is limited and it is only used for controlling the spread of MRSA. The low-rate of mupirocin-resistant *S. aureus* is due to the limited MRSA exposure to mupirocin and any subsequent development of resistance. On the other hand, the finding that mupirocin resistance is more common among *S. epidermidis* than *S. aureus* could be explained by the capacity of certain clones (i, a) to spread widely. Thus, the increased rate of mupirocin-resistant CoNS in Greece is related to the spread of methicillin-resistant epidemic hospital clones rather than the consumption of mupirocin. Measures to combat this spread, such as effective control of hospital clones, would appear to be prudent.

References

1. Cookson, B. D. (1998). The emergence of mupirocin-resistance: a challenge to infection control and antibiotic prescribing practice. *Journal of Antimicrobial Chemotherapy* **41**, 11–18.

2. Deshpande, L. M., Fix, A. M., Pfaller, M. A. *et al.* (2002). Emerging elevated mupirocin resistance rates among staphylococcal isolates in the SENTRY Antimicrobial Surveillance Program (2000): correlations of results from disk diffusion, E-test and reference dilution methods. *Diagnostic Microbiology and Infectious Diseases* **42**, 283–90.

3. Schmitz, F. J., Lindenlauf, E., Hofmann, B. *et al.* (1998). The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. *Journal of Antimicrobial Chemotherapy* **42**, 489–95.

4. National Committee for Clinical Laboratory Standards. (2000b). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically—Fifth Edition: Approved Standard M7-A5*. NCCLS, Wayne, PA, USA.

5. National Committee for Clinical Laboratory Standards. (2000a). *Performance Standards for Antimicrobial Disk Susceptibility Tests—Seventh Edition. Approved Standard M2-A7*. NCCLS, Wayne, PA, USA.

6. Perez-Roth, E., Claverie-Martin, F., Batista, N. *et al.* (2002) Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* clinical isolates in a Spanish hospital. Co-application of multiplex PCR assay and conventional microbiology methods. *Diagnostic Microbiology and Infectious Diseases* **43**, 123–8.

7. Tenover, F. C., Arbeit, R. D., Goering, R. V. *et al.* (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* **33**, 2233–9.

8. de Sousa, M. A., Bartzavali, C., Spiliopoulou, I. *et al.* (2003) Two international methicillin-resistant *Staphylococcus aureus* clones endemic in a University Hospital in Patras, Greece. *Journal of Clinical Microbiology* **41**, 2027–32

9. Spiliopoulou I., Sanches, I. S., Bartzavali, C. *et al.* (2003). Application of molecular typing methods to characterize nosocomial coagulase-negative staphylococci collected in a Greek hospital during a three-year period (1998–2000). *Microbial Drug Resistance* **9**, 261–70.

10. Maniatis, A. N., Aqel, A., Legakis, N. J. *et al.* (2001). Mupirocin resistance in *Staphylococcus aureus* from Greek hospitals. *International Journal of Antimicrobial Agents* **18**, 407–8.