J Antimicrob Chemother 2013; **68**: 1714–1717
doi:10.1093/jac/dkt085 Advance Access publication 27 March 2013

Journal of Antimicrobial Chemotherapy

Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant *Staphylococcus aureus* (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring *mupA*

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Received 4 December 2012; returned 2 January 2013; revised 12 February 2013; accepted 14 February 2013

Objectives: Mupirocin is the cornerstone of decolonization regimens, a successful strategy to prevent healthcare-associated staphylococcal infections. Several recent studies have reported alarming results: (i) an extending reservoir of *mupA*, the ancestral mobile resistance gene, among coagulase-negative staphylococci (CoNS); (ii) the emergence of a new resistance gene (*mupB*); and (iii) a growing number of mupirocin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA), including highly pathogenic clones. We performed a nationwide prospective study in France to detect such trends among invasive staphylococci.

Methods: Between October 2011 and February 2012, 367 MRSA and 708 CoNS invasive isolates were collected from 37 hospitals and analysed centrally. Mupirocin MICs were determined using the broth microdilution method. *mupA/B* PCR was performed for resistant isolates (MIC >1 mg/L). Genetic relatedness between mupirocin-resistant MRSA isolates was determined by PFGE analysis and related isolates were tested by microarray.

Results: Among MRSA isolates 2.2% ($n=8$) were classified as mupirocin resistant; 1.4% ($n=5$) showing low-level resistance (MIC ≤256 mg/L) and 0.8% ($n=3$) high-level resistance (MIC >256 mg/L). Only the latter isolates carried *mupA*. A clonal relationship was identified between two *mupA*-negative MRSA from the same hospital and three *mupA*-positive MRSA from three distant towns; these three isolates belonged to the Lyon clone. Mupirocin resistance was identified in 10.3% of CoNS, mainly highly resistant *mupA*-positive isolates (5.6%). The *mupB* gene was not detected in mupirocin-resistant MRSA or CoNS.

Conclusions: This first large national study indicates the need for thorough epidemiological monitoring and a stewardship programme to prevent and detect mupirocin resistance in staphylococci.

Keywords: Lyon clone, decolonization, invasive staphylococci

Introduction

Staphylococci are a leading cause of invasive community- and hospital-acquired infections, a significant proportion of which are considered to have an endogenous origin.[1] Decolonization strategies are recommended for collective (outbreak control) or personal (individual prevention) purposes.[1] They include cutaneous antisepsis and nasal application of a topical antimicrobial agent such as mupirocin.[1,2] Although discovered three decades ago, this specific inhibitor of isoleucyl-tRNA synthetase is still the cornerstone of nasal decontamination.[1]

Unfortunately, resistance due to mutations or acquisition of new genetic material has been reported.[2] Clinical failures during decolonization strategies were first associated with high-level mupirocin resistance mediated by *mupA*, a plasmid gene

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coding for an additional isoleucyl-tRNA synthetase for which mupirocin has no affinity.[1,2] Point mutations in the chromosomal *ileS*, inducing low-level mupirocin resistance, were then reported to lead to persistent carriage after decolonization therapy if combined with genotypic chlorhexidine resistance.[3] Because of an anticipated increase in the clinical use of mupirocin, a thorough epidemiological survey was needed to monitor the possible emergence and spread of resistant strains.[2] Three alarming reports have recently highlighted: (i) the emergence of a new mobile resistance gene (*mupB*) in methicillin-resistant *Staphylococcus aureus* (MRSA);[4] (ii) an increase in coagulase-negative staphylococci (CoNS) resistant isolates, which constitute a putative reservoir of the *mupA* resistance gene for *S. aureus*;[5] and (iii) a growing number of mupirocin-resistant MRSA, including highly pathogenic clones.[6] We therefore performed a prospective national study in France to evaluate the prevalence and genetic background of mupirocin resistance among MRSA and CoNS isolated from invasive infections.

Methods

As part of a national prospective study of invasive staphylococcal infections (Microbs study) conducted between October 2011 and February 2012, the microbiology laboratories of 37 hospitals in France (general hospitals, 9; university-affiliated hospitals, 28) collected the first 10 consecutive isolates of MRSA and 20 clinically significant isolates of CoNS from invasive infections. These isolates were centralized in the coordinating laboratory (Antoine Béclère Hospital). Invasive infections included: (i) clinically relevant bacteraemia according to the recommendations of the CDC;[7] (ii) native or device-associated osteoarticular infections; and (iii) deep soft tissue infections associated with devices such as implantable chambers or pacemakers (catheter-associated soft tissue infections were excluded). Microbiological samples were from aseptic samples, perioperative specimens and blood cultures. Only one isolate per patient was included.

Staphylococci were identified and methicillin resistance was detected in each participating laboratory. The central laboratory sent four quality control strains anonymously to each participating laboratory to evaluate its performance in identifying *S. aureus* and detecting methicillin resistance (*S. aureus* ATCC 25923, ATCC 29213 and ATCC 33591 and *Staphylococcus epidermidis* ATCC 12228).

After centralization, identification of mupirocin-resistant CoNS was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Mupirocin MICs were determined by the broth microdilution method and interpreted according to EUCAST recommendations (http://www.eucast.org/). MIC breakpoints were defined as follows: susceptible, $\leq$1 mg/L; low-level resistant, 2–256 mg/L; and high-level resistant, $\geq$512 mg/L. ATCC 25923 and ATCC 29213 (mupirocin-susceptible strains, MIC=0.5 mg/L) and *S. aureus* MUP87 (mupirocin-resistant strain MIC=1024 mg/L) were tested as control strains.[4]

All mupirocin-resistant isolates (MIC >1 mg/L) were tested for the presence of *mupA* and *mupB* genes by PCR, as previously described.[4,8] Control strains were used in each run of PCR (*S. aureus* ATCC 29213 as a negative control, *S. epidermidis* TposDEV as a *mupA*-positive control and *S. aureus* MUP87 as a *mupB*-positive control). Genetic relatedness among the mupirocin-resistant MRSA subgroup of isolates was evaluated by PFGE analysis after SmaI restriction, as previously described.[9] Additionally, the genetic background of clustered isolates was investigated by microarray assay (StaphyType Kit®, Clondiag, Jena, Germany), which allows the screening of 330 target sequences corresponding to 172 genes and their allelic variants. On the basis of the various positive and negative targets, assignments are made to clonal complex and/or sequence type and/or specific clones.[10]

Results and discussion

During the study period we included 367 MRSA and 708 CoNS isolates from the 37 participating laboratories, which were uniformly distributed geographically throughout France. The bacterial population originated from blood cultures (60%), osteoarticular specimens (29%) and other invasive samples (11%). The results of the quality control analysis showed a high level of agreement with the expected values (data not shown).

Mupirocin resistance was identified in 2.2% (8/367) of MRSA (Table 1). The MIC_{50} and MIC_{90} were both 0.25 mg/L. These mupirocin-resistant MRSA were isolated from patients with bacteraemia (n=5, associated with endocarditis, urinary tract infection, implantable venous access port infection, and skin and soft tissue infections), osteoarticular infection (n=2, including one primary bone infection and one orthopaedic implant infection) and an implantable venous access port (n=1). It is noteworthy that in 2006 we failed to identify any phenotypic mupirocin resistance in a national sample of MRSA isolates from community-acquired skin and soft tissue infections (n=34).[11] In 2007, 2% (1/51) of MRSA strains isolated from bloodstream infections were mupirocin resistant.[9] The proportion of high-level mupirocin-resistant MRSA in France (0.8%) seems to be in the same range as in hospitals in the USA (0.62%) but lower than in Canada or China (7%).[12,13]

Recently, Lee *et al.*[3] reported the putative role in persistent MRSA carriage of low-level mupirocin resistance combined with *qacA/B* genes, which are putative elements for chlorhexidine resistance. In the present work, *mupA* was identified in the three isolates exhibiting a high level of resistance (Table 1). No mupirocin-resistant isolate harboured the *mupB* gene, a new resistance gene that must be screened for so as to quickly detect any emerging phenomenon. The eight mupirocin-resistant MRSA isolates originated from seven different hospitals. Two of the five low-level mupirocin-resistant isolates were closely related by PFGE (data not shown) and originated from the same hospital. This finding probably indicates a cross-transmission inside this healthcare facility. The three high-level resistant *mupA*-positive MRSA isolates originated from three distant towns (Lyon, Orléans and Toulouse) and were indistinguishable or closely related (Figure 1). The microarray assay assigned them to the Lyon clone, which is the most prevalent invasive clone in France.[14] Interestingly, the emergence of Lyon clone MRSA isolates harbouring *mupA* has been recently reported in England (A. Kearns, HPA, personal communication). If confirmed, the spread through European countries of such a mupirocin-resistant MRSA clone well adapted to humans could hinder preventive strategies using mupirocin ointment. Furthermore, McDougal *et al.*[6] have reported the dissemination of MRSA with different PFGE patterns but harbouring the same plasmid carrying *mupA*. Such horizontal plasmid transfer should be screened for in France.

Mupirocin resistance was identified in 10.3% (73/708) of CoNS. The MIC_{50} and MIC_{90} were 0.25 mg/L and 4 mg/L, respectively. These mupirocin-resistant CoNS were identified in 29 of 37 centres, mainly as *S. epidermidis* (95%) and methicillin-resistant isolates (84.9%). They originated from patients with bacteraemia (n=46, mostly associated with central venous catheter infection) and invasive infections (n=27, mostly orthopaedic implant infections). The majority of mupirocin-resistant CoNS isolates showed a high level of resistance and a *mupA* PCR-positive

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Table 1. Prevalence of mupirocin resistance including low- and high-level resistance, ranges of mupirocin MICs and *mupA/B* PCR results among MRSA and CoNS

	MRSA				CoNS			
	total	isolates with an MIC ≤1 mg/L	isolates with an MIC >1 and ≤256 mg/L[a]	isolates with an MIC ≥512 mg/L[b]	total	isolates with an MIC ≤1 mg/L	isolates with an MIC >1 and ≤256 mg/L[a]	isolates with an MIC ≥512 mg/L[b]
n (%)	367 (100)	359 (97.8)	5 (1.4)	3 (0.8)	708 (100)	635 (89.7)	33 (4.7)	40 (5.6)
Range, mg/L	0.03–1024	0.03–1	2–64	1024	0.03–1024	0.03–1	2–256	512–1024
mupA PCR+, *n* (%)	ND	ND	0	3 (0.8)	ND	ND	6 (0.8)	40 (5.6)
mupB PCR+, *n* (%)	ND	ND	0	0	ND	ND	0	0

ND, not determined; PCR+, positive PCR signal.
[a]Low level of resistance.
[b]High level of resistance.

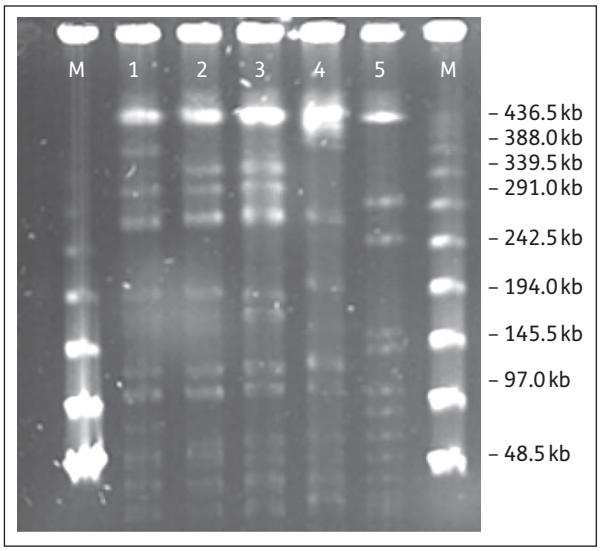

Figure 1. PFGE patterns of the mupirocin-resistant MRSA isolates. Lanes 1 to 3, high-level resistant *mupA*-positive MRSA strains; lane 4: susceptible *mupA*-negative MRSA control strain; lane 5, *S. aureus* ATCC 25923; M, molecular weight marker.

signal (Table 1). Nonetheless, 18.2% (6/33) of the low-level mupirocin-resistant CoNS still carried *mupA*. This disturbing finding has previously been reported to be associated with the chromosomal location of the gene.[2] The prevalence of *mupA*-harbouring CoNS reached 6.5% (46/708), a value close to the 8% reported by Bathoorn *et al.*[5] in 2006. No *mupB*-positive CoNS were detected.

To the best of our knowledge ours is the first national point prevalence study of phenotypic and genotypic mupirocin resistance among CoNS to include screening for *mupB*. In France, mupirocin is available both for nasal decontamination and topical therapy of skin and soft tissue infections. The use of mupirocin will obviously increase in the years to come.[2] In the present study, we focused on invasive isolates and did not consider methicillin-susceptible *S. aureus* isolates. Additional data are needed in other settings, such as nasal carriage.

Conclusions

We provide recent and nationwide data about emerging threats in the field of mupirocin resistance. We highlight the spread of a high-level resistant *mupA*-harbouring MRSA clone that belongs to the most frequent invasive clone in France. If confirmed at a European level this finding may represent a worrying development for preventive strategies such as nasal decolonization. Additionally, we identified a huge reservoir of *mupA* among CoNS. Our findings are strong arguments in favour of a national mupirocin stewardship programme, associated with careful monitoring of resistance in both *S. aureus* and CoNS.

Acknowledgements

We would like to thank Roberto G. Melano from the Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, for the control strain. This work was presented in part at the Thirty-second Interdisciplinary Meeting of Anti-Infective Chemotherapy (RICAI), Paris, France, 2012 (Poster no. P359).

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Funding

This work was partially funded by a grant from Novartis, France.

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Transparency declarations

None to declare.

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