Relationship between molecular epidemiology and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in a French teaching hospital

Michelle Thouverez,[1] Arno Muller,[1] Didier Hocquet,[2] Daniel Talon[1] and Xavier Bertrand[1]

Service d'Hygiène Hospitalière et d'Epidémiologie Moléculaire[1] and Service de Bactériologie[2], Centre Hospitalier Universitaire Jean Minjoz, Besançon, France

Correspondence
Daniel Talon
daniel.talon@ufc-chu.univ-fcomte.fr

Received 10 March 2003
Accepted 23 May 2003

The objective of this study was to investigate the relationship between molecular epidemiology and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) over a period of 4 years. The antibiotype of all MRSA isolates that were identified during a yearly period of 3 months was determined; 50 consecutive non-replicate MRSA isolates were typed each year. Susceptibility rates to gentamicin, tobramycin and ofloxacin remained stable (95, 16 and 4 %, respectively). In contrast, the proportion of MRSA isolates susceptible to erythromycin increased progressively from 10·5 to 32·5 % ($P < 0{\cdot}001$). PFGE analysis of genomic DNA from 200 isolates revealed the presence of 15 different clones. Two epidemic clones were identified, which contained 150 (clone A) and 28 (clone C) isolates. Non-epidemic strains were more frequently susceptible to ofloxacin (31·8 versus 1·1 %) and tobramycin (45·4 versus 16·8 %) than epidemic strains; those isolates that were susceptible to all antibiotics tested belonged to sporadic clones. The increase of erythromycin susceptibility within MRSA isolates was caused by the emergence of clone C. This study suggests that when selection pressure exerted by an antibiotic is insufficient (i.e. below a threshold level), fitness advantages play a predominant role in the dissemination of MRSA clones. The balance between the selection pressure exerted by antibiotics and the disadvantage of lower replication rates of resistant strains in the absence of antibiotics complicates the biological model of clonal dissemination of epidemic MRSA strains.

INTRODUCTION

Over the last four decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has spread throughout the world and become highly endemic in many geographical areas. This pathogen causes severe morbidity and mortality in hospitals worldwide (Boyce, 1990; Voss *et al.*, 1994; Aubry-Damon *et al.*, 1997; Bertrand *et al.*, 2000). In France, 30–40 % of *S. aureus* strains are methicillin-resistant and the incidence of MRSA isolated from clinical specimens ranges from 0·04 to 3·6 per 1000 hospital-days, according to the hospital. The overall incidence of MRSA is approximately 0·8 per 1000 hospital-days (Albertini *et al.*, 2002). All MRSA strains isolated before 1990 were homogeneously resistant to methicillin and also resistant to an increasing number of major classes of antibiotics, including aminoglycosides, fluoroquinolones and macrolides. In 1991, gentamicin-susceptible (GS) MRSA clones emerged in a number of Parisian hospitals (Aubry-Damon *et al.*, 1997; Lemaître *et al.*, 1998) and subsequently spread to our region in eastern France in 1994 (Bertrand *et al.*, 2000). In our hospital, the prevalence of tobramycin- and amikacin-susceptible MRSA has increased since 1996 (Talon *et al.*, 2002) and the prevalence of erythromycin-susceptible MRSA has increased since 1998. Nowadays, it is common for MRSA isolates to be susceptible to all non-β-lactam antibiotics tested (except fluoroquinolones).

To improve our understanding of changes in MRSA susceptibility to non-β-lactam antibiotics, we evaluated the relationship between antibiotic susceptibility and MRSA genotype over a 4-year period. For this purpose, we used PFGE as an epidemiological tool.

Abbreviations: DDD, defined daily dose; GR, gentamicin-resistant; GS, gentamicin-susceptible; MRSA, methicillin-resistant *Staphylococcus aureus*.

METHODS

Setting. Besançon is the largest city (130 000 inhabitants) in Franche-Comté, a region of eastern France with approximately one million

inhabitants. Besançon Hospital is a university-affiliated hospital with 1219 acute-care beds, divided into 59 units (35 medical units, 21 surgical units and three intensive-care units). Specialty services include cardiothoracic surgery and organ and bone-marrow transplantation. Approximately 50 000 inpatients are admitted each year, for a total of 350 000 patient-days.

In 1994, our infection-control committee made the control of MRSA a major priority. An MRSA control programme was implemented progressively in all high-risk units. Since 1997, 14 of the 59 units (corresponding to all high-risk departments, including the adult intensive-care units and the septic surgical unit) have been following this programme. The control strategy is based on screening for MRSA on admission: nasal fluid samples are obtained by using sterile cotton swabs from all patients on admission. In the first 48 h after admission, before the results of the screening cultures are available, patients are considered to be positive for MRSA and kept in isolation. Patients who are actually positive for MRSA are then given nasal mupirocin. All MRSA-positive patients are kept in individual rooms or in cohorts. Special precautions are taken to prevent cross-contamination, including the use of disposable gowns and gloves, hand-washing, alcohol hand-rubbing and the implementation of strict environmental hygiene measures. These procedures are also applied to patients in low-risk departments who give clinical samples that test positive for MRSA, who are not screened on admission. The programme does not include any restrictions on antibiotic use.

Bacterial isolates. Since 1999, our hospital has participated in an MRSA surveillance network. This network carries out annual 3-month studies (April–June). During this period, all MRSA strains that were isolated for diagnostic purposes (excluding surveillance specimens) from patients hospitalized for >24 h were collected and kept at −73 °C. When multiple MRSA isolates were obtained from the same patient, only the first isolate was included; duplicate isolates were excluded.

Antimicrobial agent susceptibility testing. The antibiotype of all MRSA isolates identified during the surveillance periods was determined. Antibiotic susceptibility was determined by the disc-diffusion method on Mueller–Hinton agar with the following discs (Bio-Rad): gentamicin (10 IU), tobramycin (10 μg), kanamycin (30 IU), erythromycin (15 IU), clindamycin (2 IU), pristinamycin (15 μg), ofloxacin (5 μg), fusidic acid (10 μg) and rifampicin (30 μg). Isolates were classified as susceptible, intermediate or resistant, according to the criteria of the Antibiogram Committee of the French Society for Microbiology (Soussy *et al.*, 2000). Oxacillin resistance was tested by incubating isolates with a 5 μg oxacillin disc at 30 °C for 48 h. Susceptibility to glycopeptides was tested by spotting 10 μl suspension that contained 10^6 c.f.u. ml^{-1} onto Mueller–Hinton agar (Bio-Rad) plates that contained 4 or 16 mg teicoplanin l^{-1}.

Molecular typing. Fifty consecutive, non-replicate isolates that were collected each year during the study period (of 280–300 MRSA recovered per year, excluding surveillance samples) were typed. The macrorestriction pattern of total DNA was determined by PFGE (CHEF-DR III; Bio-Rad) using *Sma*I, as described previously by Prevost *et al.* (1992). GelCompar software (Applied Maths) was used to establish a DNA similarity matrix based on the Dice coefficient (2×2 strain comparisons). A dendrogram was constructed by using the UPGMA clustering method with the Dice coefficient. To ensure that the gels were comparable, *S. aureus* NCTC 8325 was included as a reference strain. Isolates with indistinguishable PFGE patterns were assigned to the same clone and clonal variant. Strains that differed by up to (and including) six bands were considered to belong to different clonal variants; strains that differed by more than six bands were considered to belong to different clones (Tenover *et al.*, 1995). Clones and clonal variants were designated by letters and by numbers in suffix, respectively.

Antibiotic use. Annual quantities of antibiotics delivered to each unit during the study period were collected from the pharmacy information system. Grams and international units of antimicrobials were further converted into defined daily doses (DDDs) (Natsch *et al.*, 1998).

RESULTS

MRSA endemicity

Table 1 reports the frequency of MRSA in our hospital since 1994, based on data collected as part of the MRSA surveillance programme (1999–2002). Data collected in 1994 and 1998 were obtained with the same methodology.

Susceptibility to antimicrobials

Pooled data for the last 4 years (Table 2) show that since 1999, 95 % of MRSA isolates in our hospital were gentamicin-susceptible and that approximately 15 % of MRSA isolates were susceptible to tobramycin. The proportion of MRSA susceptible to erythromycin increased significantly and progressively from 7·0 to 32·5 % during the study period ($P < 0{\cdot}001$). In contrast, the isolates remained resistant to ofloxacin and susceptible to rifampicin and fusidic acid. No isolates exhibited reduced susceptibility to teicoplanin (MIC > 4 mg l^{-1}).

Despite the change in antibiotic-resistance patterns in MRSA, when four antimicrobial agents (gentamicin, tobramycin, erythromycin and ofloxacin) were used as phenotypic markers, 61·4 % of MRSA isolates displayed the major antibiotype (1d) (Table 3). Approximately one-sixth (15·4 %) of the isolates belonged to antibiotype 1c and 12·8 % belonged to antibiotype 2d.

Molecular typing

PFGE analysis of genomic DNA from 200 isolates revealed 15 different clones. Two epidemic clones were identified, which contained 150 (clone A) and 28 (clone C) isolates (Fig. 1). Four other clones that contained two, two, four and five isolates were called micro-epidemic, and nine sporadic clones each contained a single isolate. Clone A was divided

Table 1. Epidemiology of MRSA in Besançon Hospital

Year	Incidence of MRSA per 1000 hospital-days*	Frequency of methicillin resistance among *S. aureus* isolates (%)
1994	1·40	30·3
1998	1·17	24·9
1999	1·04	21·1
2000	0·83	22·8
2001	0·89	25·8
2002	0·92	24·8

*MRSA isolates from clinical specimens from patients hospitalized for >24 h.

Table 2. Evolution of MRSA resistance to non-β-lactam antibiotics in Besançon Hospital from 1999 to 2002

Values are percentages of MRSA isolates that are susceptible to each antibiotic. NT, Not tested.

Antibiotic	1999 ($n = 86$)	2000 ($n = 77$)	2001 ($n = 76$)	2002 ($n = 80$)	Total ($n = 319$)	P*
Gentamicin	93·0	100	89·5	96·3	94·7	0·999
Tobramycin	18·6	19·5	13·2	12·5	16·0	0·179
Kanamycin	18·6	19·5	13·2	13·8	16·3	0·50
Erythromycin	7·0	20·8	21·1	32·5	20·1	$< 0·001$
Clindamycin	10·5	23·4	23·7	30·0	21·6	0·0032
Pristinamycin	94·2	98·7	93·4	95·0	95·3	0·825
Ofloxacin	4·7	5·2	4·0	2·5	4·1	0·437
Fusidic acid	82·6	88·3	85·5	86·3	85·6	0·610
Rifampicin	87·2	92·2	92·1	90·0	90·3	0·552
Teicoplanin	100	100	100	100	100	NT
Vancomycin	100	100	100	100	100	NT

*χ^2 test for trend in proportions (Epi Info software, version 6.1). $P < 0·05$ was considered to be significant.

Table 3. Resistance patterns of 319 MRSA isolates identified between 1999 and 2002

Pattern no.	Susceptibility to aminoglycosides*	Resistance to other antibiotics†	1999 ($n = 86$)	2000 ($n = 77$)	2001 ($n = 76$)	2002 ($n = 80$)	Frequency‡
1	GS, TR		64	62	58	67	NA
1a		None	2	1	1	–	1·2
1b		Ery	1	2	–	–	0·9
1c		Oflo	2	10	15	22	15·4
1d		Ery, Oflo	59	49	43	45	61·4
2	GS, TS		16	15	10	10	NA
2a		None	1	1	1	2	1·6
2b		Ery	–	–	1	–	0·3
2c		Oflo	–	4	–	2	1·9
2d		Ery, Oflo	15	10	8	8	12·8
3	GR, TR		6	0	8	3	NA
3a		Oflo	1	0	–	–	0·3
3b		Ery, Oflo	5	–	8	3	5·0

*GS, Gentamicin-susceptible; GR, gentamicin-resistant; TS, tobramycin-susceptible; TR, tobramycin-resistant.

†Ery, Erythromycin; Oflo, ofloxacin.

‡Values are percentages of total isolates. NA, Not applicable.

into 28 clonal variants (A_1–A_{28}) and clone C was divided into 14 clonal variants (C_1–C_{14}). Clone A also included one of the isolates collected in Pitié-Salpêtrière Hospital (Paris, France) in 1991 [called clone A by Lemaître *et al.* (1998)] and three strains that belong to the major clone in our region, which were isolated in 1998 (Bertrand *et al.*, 2000). Clone C also included an isolate collected in Pitié-Salpêtrière Hospital in 1991 [called clone C by Lemaître *et al.* (1998)]. Clone C was not isolated in our hospital in 1998 (Bertrand *et al.*, 2000); it was first detected in our hospital in 1999 and accounted for 22 % of MRSA in 2002 (Table 4). The proportion of MRSA isolates that belonged to clone A decreased progressively during the study period (from 86 to 66 %). The frequency of non-epidemic isolates remained stable (10–12 %). However, the overall incidence of MRSA in our hospital increased slightly in 2001–2002 (Table 1).

Association between antibiotic susceptibility and genotype

Association between clone and antibiotype is reported in Table 4. Non-epidemic strains were more frequently suscep-

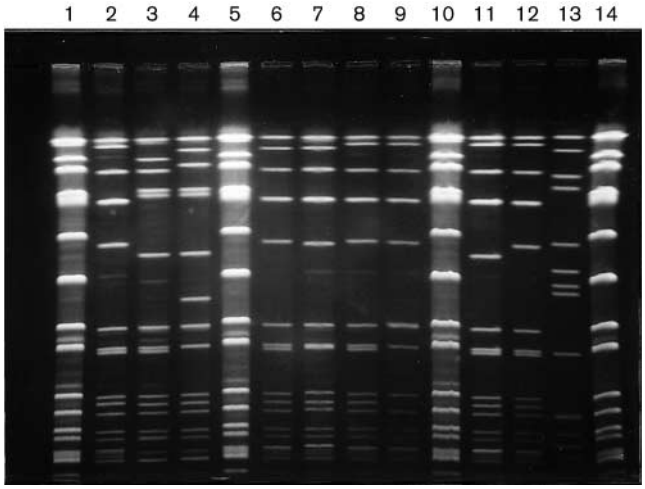

Fig. 1. *Sma*I-restricted genomic DNA of MRSA isolates and reference strain. Lanes 1, 5, 10 and 14, *Sma*I-restricted genomic DNA of *S. aureus* NCTC 8325; 2, isolate 40219/01 (clone A, variant A_1); 3, isolate 39561/01 (clone C, variant C_4); 4, isolate 40513/01 (clone C, variant C_1); 6, isolate 41281/01 (clone A, variant A_8); 7, isolate 41692/01 (clone A, variant A_9); 8, 42210/01 (clone A, variant A_{10}); 9, isolate 43565/01 (clone A, variant A_{11}); 11, isolate 43566/01 (clone A, variant A_{14}); 12, isolate 44500/01 (clone A, variant A_{10}); 13, isolate 46483 (sporadic clone).

Table 4. Association between clone and antibiotic markers

Values are percentages of isolates susceptible to each antibiotic, with number of isolates (*n*) in parentheses.

Antibiotic	Epidemic clones		Other clones	
	Clone A (*n* = 150)	Clone C (*n* = 28)	Micro-epidemic (*n* = 13)	Sporadic (*n* = 9)
Gentamicin	94·7 (142)	96·4 (27)	100 (13)	88·8 (8)
Tobramycin	18·0 (27)	10·7 (3)	46·1 (6)	44·4 (4)
Erythromycin	2·7 (4)	78·6 (22)	53·8 (7)	55·5 (5)
Ofloxacin	0·7 (1)	3·6 (1)	23·1 (3)	44·4 (4)

tible to ofloxacin and tobramycin than epidemic clones. The four isolates that were susceptible to all antibiotics tested belonged to sporadic clones. Nine of the ten gentamicin-resistant (GR) MRSA isolates belonged to epidemic clones (eight to clone A and one to clone C) and were not related to the epidemic GR-MRSA clones that were present in our hospital in the early 1990s (data not shown). Moreover, the eight isolates clustered in the major epidemic clone were distributed between seven clonal variants. Both epidemic and non-epidemic strains were susceptible to tobramycin; tobramycin-susceptible isolates were distributed in numerous clonal variants of the epidemic clones. The increase in erythromycin susceptibility was caused by the wide-scale dissemination of clone C. Indeed, erythromycin-susceptible MRSA remained very rare in clone A (4 %) but accounted for 78·6 % of isolates in clone C. Moreover, the frequency of erythromycin susceptibility increased in clone C during the study period: 20, 60 and 90·9 % in 2000, 2001 and 2002, respectively.

Antibiotic use

Table 5 reports the evolution of antibiotic use from 1999 to 2002 and the distribution of antibiotic classes among the total antimicrobial use. This distribution did not vary during the study period and total antibiotic use remained stable.

DISCUSSION

We might be able to improve infection-control practices if we can understand dynamic changes in MRSA clonal dissemination and factors that influence competition between several clones. Many studies have been carried out on MRSA in France and have clearly established the epidemiological picture of this pathogen (Aubry-Damon *et al.*, 1997; Lemaître *et al.*, 1998; Lelièvre *et al.*, 1999; Bertrand *et al.*, 2000; Galdbart *et al.*, 2000).

The proportion of MRSA isolates that is susceptible to gentamicin increased dramatically between 1994 and 1998 (Bertrand *et al.*, 2000). Currently, 95 % of MRSA isolates from our hospital are gentamicin-susceptible (GS-MRSA). Several reports support the hypothesis that GS-MRSA reemerged due to the spread of GR-MRSA clones in which the *aac6′–aph2″* gene had been excised or deleted (Lemaître *et al.*, 1998; Lelièvre *et al.*, 1999; Blanc *et al.*, 2001). The fitness of these clones probably increased during their evolution, giving them an advantage over other clones. Experiments *in vitro* have shown that GS-MRSA isolates grow more rapidly than GR-MRSA isolates, thus giving them a competitive advantage (Bertrand *et al.*, 2000; Laurent *et al.*, 2001). The switch from GR- to GS-MRSA clones has been attributed to changes in antibiotic use (Aubry-Damon *et al.*, 1997; Lelièvre *et al.*, 1999). However, this hypothesis has not been confirmed and aminoglycoside use does not appear to play a major role in the dissemination of GS-MRSA strains (Bertrand *et al.*, 2000; Blanc *et al.*, 2001). In 1998, clone A was divided into seven clonal variants and did not include GR-MRSA. In our study, the genomic diversity within this clone appeared to be greater (28 clonal variants). The presence of GR-MRSA in this clone does not reflect the reemergence of ancestral clones but merely the evolution of GS-MRSA through the acquisition of a gene (perhaps from coagulase-negative staphylococci) that confers resistance to gentamicin.

Tobramycin-susceptible MRSA isolates were rare until 1998 (Bertrand *et al.*, 2000) but became more frequent in 1999 (Talon *et al.*, 2002). Susceptibility to tobramycin appears to be a phenotypic variation among MRSA isolates. The unpredictability of this modification is supported by the fact that it occurred within various clones and clonal variants, regardless of their epidemic nature. This modification did

Table 5. Antibiotic use in Besançon Hospital, 1999–2002

Values are expressed as DDDs, with percentage in parentheses.

Antibiotic class	Antibiotic use			
	1999	2000	2001	2002
β-Lactam	164 530·0 (71·2)	182 138·0 (72·1)	189 202·0 (72·3)	182 348·0 (71·4)
Quinolone	29 668·3 (12·8)	34 167·9 (13·5)	37 200·0 (14·2)	37 762·7 (14·8)
Aminoglycoside	9 823·3 (4·3)	9 233·1 (3·7)	9 484·0 (3·6)	9 052·8 (3·5)
Macrolide	13 016·7 (5·6)	13 201·3 (5·2)	11 804·3 (4·5)	12 137·7 (4·8)
Glycopeptide	10 031·2 (4·3)	8 789·2 (3·5)	9 226·9 (3·5)	9 497·3 (3·7)
Other	3 961·8 (1·7)	5 210·8 (2·1)	4 780·1 (1·8)	4 516·7 (1·8)
Total	231 031·3	252 740·3	261 697·3	255 315·2

not confer any advantages in terms of fitness (Bertrand *et al.*, 2000). The emergence of tobramycin susceptibility within MRSA does not seem to be related to variations in aminoglycoside use. Indeed, it emerged in parallel in various settings, even though the selective pressure exerted by aminoglycosides was considerably different (Talon *et al.*, 2002).

The increased frequency of erythromycin susceptibility among MRSA isolates was due to the dissemination of a new clone (C) in our hospital. This clone was described by Lemaître *et al.* (1998) as being a minor clone that was present in a Parisian hospital in 1992. The frequency of clone C in this hospital increased considerably between 1992 and 1996. This clone, which was probably introduced into our hospital via a transferred patient, presented a similar epidemiology in our hospital 8 years later. Thus, the evolution of erythromycin susceptibility among MRSA isolates is a strong marker of the dissemination of clone C. This surveillance is particularly important because it seems that clone C disseminates without competing with the major epidemic clone, A. The emergence of clone C was probably responsible for the slight increase in MRSA incidence in our hospital that was first observed in 2001 and confirmed in 2002, although the incidence of MRSA had been decreasing since the MRSA control programme was initiated in 1994 (Bailly *et al.*, 1999; Bertrand *et al.*, 2000).

In our hospital, there were no restrictions on antibiotic use. During the study period, no significant variations in antibiotic usage occurred (Table 5). β-Lactams and fluoroquinolones accounted for 71·8 and 13·9 %, respectively, of all antibiotics used. Thus, these two classes of antimicrobial exerted considerable selection pressure for resistant bacteria such as MRSA. It is noteworthy that the ofloxacin susceptibility level remains extremely low in our hospital; the loss of ofloxacin resistance seems to prevent the dissemination of such strains (Table 4). It is possible that when the selection pressure exerted by an antibiotic is insufficient (i.e. below a threshold level), fitness advantages play a predominant role in the dissemination of MRSA clones. Therefore, the concept of 'total use threshold', introduced by Levy (1994), appears to be an important factor in the antibiotic-resistance changes in epidemic MRSA. In our case, the use of aminoglycosides and macrolides (3·7 and 5·0 % of total antibiotic use, respectively) was below this threshold, thus variations in use of both antibiotics did not affect the evolution of the MRSA susceptibility pattern.

In other countries such as Germany (Witte *et al.*, 2001), Finland (Salmenlinna & Vuopio-Varkila, 2001), Greece (Polyzou *et al.*, 2001; Pournaras *et al.*, 2001) and Australia (Torvaldsen *et al.*, 1999), a similar trend of emergence and spread of MRSA with susceptibility to aminoglycosides, macrolides and other antibiotics has been observed.

The spread of a new epidemic MRSA clone in our hospital is a matter of concern because it could reverse the downward trend of MRSA incidence observed since 1994. At a practical clinical level, continuing evolution of MRSA provides an opportunity for the controlled reintroduction of antibiotics (particularly aminoglycosides) in anti-MRSA therapies, meaning that we would no longer be reliant on glycopeptides. The balance between selection pressure exerted by antibiotics and the disadvantage of lower replication rates of resistant strains in the absence of antibiotics complicates the biological model of the clonal dissemination of epidemic MRSA strains. Further studies, including *in vitro* fitness analysis and epidemiological surveys, should be undertaken to determine the relative effects of antibiotic use and bacterial fitness on MRSA epidemiology.

REFERENCES

Albertini, M. T., Benoit, C., Berardi, L. & 25 other authors (2002). Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterobacteriaceae* producing extended-spectrum beta-lactamase (ESBLE) in Northern France: a five-year multicentre incidence study. The Microbiology Surveillance Network of Northern France. *J Hosp Infect* **52**, 107–113.

Aubry-Damon, H., Legrand, P., Brun-Buisson, C., Astier, A., Soussy, C. J. & Leclercq, R. (1997). Reemergence of gentamicin-susceptible strains of methicillin-resistant *Staphylococcus aureus*: roles of an infection control program and changes in aminoglycoside use. *Clin Infect Dis* **25**, 647–653.

Bailly, P., Mulin, B., Minary, P. & Talon, D. (1999). Contrôle des infections à *Staphylococcus aureus* méticillino-résistant dans un CHU:

analyse critique des résultats obtenus. Le Réseau Franc-Comtois de Lutte contre les Infections Nosocomiales. *Med Mal Infect* **29**, 178–183 (in French).

Bertrand, X., Thouverez, M. & Talon, D. (2000). Antibiotic susceptibility and genotypic characterization of methicillin-resistant *Staphylococcus aureus* strains in eastern France. *J Hosp Infect* **46**, 280–287.

Blanc, D. S., Francioli, P., Le Coustumier, A., Gazagne, L., Lecaillon, E., Gueudet, P., Vandenesch, F. & Etienne, J. (2001). Reemergence of gentamicin-susceptible strains of methicillin-resistant *Staphylococcus aureus* in France: a phylogenetic approach. *J Clin Microbiol* **39**, 2287–2290.

Boyce, J. M. (1990). Increasing prevalence of methicillin-resistant *Staphylococcus aureus* in the United States. *Infect Control Hosp Epidemiol* **11**, 639–642.

Galdbart, J.-O., Morvan, A. & El Solh, N. (2000). Phenotypic and molecular typing of nosocomial methicillin-resistant *Staphylococcus aureus* strains susceptible to gentamicin isolated in France from 1995 to 1997. *J Clin Microbiol* **38**, 185–190.

Laurent, F., Lelièvre, H., Cornu, M., Vandenesch, F., Carret, G., Etienne, J. & Flandrois, J. P. (2001). Fitness and competitive growth advantage of new gentamicin-susceptible MRSA clones spreading in French hospitals. *J Antimicrob Chemother* **47**, 277–283.

Lelièvre, H., Lina, G., Jones, M. E. & 9 other authors (1999). Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. *J Clin Microbiol* **37**, 3452–3457.

Lemaître, N., Sougakoff, W., Masmoudi, A., Fievet, M.-H., Bismuth, R. & Jarlier, V. (1998). Characterization of gentamicin-susceptible strains of methicillin-resistant *Staphylococcus aureus* involved in nosocomial spread. *J Clin Microbiol* **36**, 81–85.

Levy, S. B. (1994). Balancing the drug-resistance equation. *Trends Microbiol* **2**, 341–342.

Natsch, S., Hekster, Y. A., de Jong, R., Heerdink, E. R., Herings, R. M. & van der Meer, J. W. (1998). Application of the ATC/DDD methodology to monitor antibiotic drug use. *Eur J Clin Microbiol Infect Dis* **17**, 20–24.

Polyzou, A., Slavakis, A., Pournaras, S., Maniatis, A. N., Sofianou, D. & Tsakris, A. (2001). Predominance of a methicillin-resistant *Staphylococcus aureus* clone susceptible to erythromycin and several other non-β-lactam antibiotics in a Greek hospital. *J Antimicrob Chemother* **48**, 231–234.

Pournaras, S., Slavakis, A., Polyzou, A., Sofianou, D., Maniatis, A. N. & Tsakris, A. (2001). Nosocomial spread of an unusual methicillin-resistant *Staphylococcus aureus* clone that is sensitive to all non-β-lactam antibiotics, including tobramycin. *J Clin Microbiol* **39**, 779–781.

Prevost, G., Jaulhac, B. & Piemont, Y. (1992). DNA fingerprinting by pulsed-field gel electrophoresis is more effective than ribotyping in distinguishing among methicillin-resistant *Staphylococcus aureus* isolates. *J Clin Microbiol* **30**, 967–973.

Salmenlinna, S. & Vuopio-Varkila, J. (2001). Recognition of two groups of methicillin-resistant *Staphylococcus aureus* strains based on epidemiology, antimicrobial susceptibility, hypervariable-region type, and ribotype in Finland. *J Clin Microbiol* **39**, 2243–2247.

Soussy, C. J., Carret, G., Cavallo, J. D. & 15 other authors (2000). Antibiogram Committee of the French Microbiology Society. Report 2000-2001. *Pathol Biol* **48**, 832–871 (in French).

Talon, D., Delière, E. & Bertrand, X. (2002). Characterization of methicillin-resistant *Staphylococcus aureus* strains susceptible to tobramycin. *Int J Antimicrob Agents* **20**, 174–179.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.

Torvaldsen, S., Roberts, C. & Riley, T. V. (1999). The continuing evolution of methicillin-resistant *Staphylococcus aureus* in Western Australia. *Infect Control Hosp Epidemiol* **20**, 133–135.

Voss, A., Milatovic, D., Wallrauch-Schwarz, C., Rosdahl, V. T. & Braveny, I. (1994). Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* **13**, 50–55.

Witte, W., Braulke, C., Cuny, C., Heuck, D. & Kresken, M. (2001). Changing pattern of antibiotic resistance in methicillin-resistant *Staphylococcus aureus* from German hospitals. *Infect Control Hosp Epidemiol* **22**, 683–686.