

Decreased susceptibility to glycopeptides in methicillin-resistant *Staphylococcus aureus*: a 20 year study in a large French teaching hospital, 1983–2002

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Objectives: To assess the evolution of glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in a large French teaching hospital from 1983 to 2002.

Methods: Determination of glycopeptide MICs by using the Etest method in Mueller–Hinton agar on a sample of randomly selected MRSA strains.

Results: A total of 1445 MRSA strains were tested, and one vancomycin-intermediate MRSA (VISA) and 31 teicoplanin-intermediate MRSA (TISA) strains were detected. The first strains were detected in 1985, and all strains were gentamicin resistant (GR). None of the gentamicin-susceptible strains had a glycopeptide MIC > 3 mg/L. In addition, there was a significant increase in glycopeptide MIC geometric means over the years, and this increase was higher for teicoplanin than for vancomycin.

Conclusions: The higher increase in teicoplanin MICs, and the good correlation between vancomycin and teicoplanin MICs, suggests systematic determination of teicoplanin MIC to screen for abnormal glycopeptide susceptibility among GR-MRSA.

Keywords: *S. aureus*, vancomycin, teicoplanin, GISA, gentamicin

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is highly prevalent in French hospitals as it is in those of other industrialized countries such as the USA, the UK, Italy and Spain.¹ Until recently, many hospital physicians did not consider MRSA as a major medical threat because glycopeptides remain active despite intensive use over the past 30 years. However, clinical strains of MRSA with reduced susceptibility to vancomycin or more generally to glycopeptides, and referred as to VISA or GISA, have been reported in several countries.^{2–5} These reports, mainly from countries where MRSA is highly prevalent, alarmed the medical community and the apocalypse was foreseen.⁶ It turned out that these strains were present in some hospitals for longer than assumed and that no major diffusion of a single clone was observed as compared with epidemic MRSA.⁷

MIC determination in Mueller–Hinton (MH) media is the most widely accepted method to define GISA.^{8–10} Some authors proposed the use of a second test to confirm the resistance. However, this has not been presently approved by NCCLS. Hetero-GISA, i.e. strains with a subpopulation able to grow in presence of glycopeptide compounds, can be detected by specific methods

that are not well standardized.^{11–13} In addition, the clinical significance of hetero-GISA is still controversial. This is the reason why MIC determination is the method of choice to survey the occurrence of MRSA strains with decreased susceptibility to glycopeptides.

In this context, we performed a retrospective study to assess the evolution of glycopeptide susceptibility of MRSA strains during the last 20 years in a large teaching hospital. The aim was to identify the year of first occurrence of GISA and to assess the magnitude of the phenomenon.

Materials and methods

Pitié-Salpêtrière hospital is a 2000 bed teaching hospital located in Paris. All MRSA strains isolated from clinical samples have been systematically stored for more than 20 years. This MRSA strain collection was used to randomly sample at least 100 strains for every uneven year from 1983 to 2001 and also for the year 2002. Strains were removed from storage, streaked on BHI agar and incubated for 24 h at 35°C under aerobic conditions. Because some strains did not grow after storage removal, the numbers of strains tested for each year was unequal and sometimes lower than a hundred.

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Control strains included the reference strain ATCC 25923, and Mu3 and Mu50 strains kindly provided by K. Hiramatsu.

Recommendations of the Comité de l'Antibiogramme from the French Society for Microbiology (CA-SFM) for susceptibility testing and interpretation of results were followed.¹⁰ In brief, MH agar was used for glycopeptide MIC determination by Etest method (AB Biodisk, Sweden) and disc diffusion test for gentamicin susceptibility. Etest strips were deposited on to an MH agar surface that had been previously overlaid with bacterial suspensions calibrated as 0.5 McFarland (10^6 cfu/mL) by using a rotating turn-table (Retro C80TM, AB Biodisk, Sweden). Plates were incubated for a full 24 h at 37°C before reading. Reading was performed in duplicate independently by two persons, according to the Etest supplier's guidelines.

Breakpoints used for interpretative criteria were those recommended by the NCCLS⁹ (≤ 4 mg/L and ≥ 32 mg/L for vancomycin, and ≤ 8 mg/L and ≥ 32 mg/L for teicoplanin), and by the CA-SFM¹⁰ (≤ 4 mg/L and ≥ 16 mg/L for both glycopeptides).

Results

A total of 1445 MRSA strains were selected for MIC determination, including 1075 (74.4%) gentamicin-resistant (GR) strains and 370 (25.6%) gentamicin-susceptible (GS) strains. GS-MRSA

strains were seldom isolated in our hospital before 1993, but they progressively replaced GR-MRSA after the year 1993 and became predominant after 1997. Therefore, more GS-MRSA strains were included in the study after 1993.

The distribution of glycopeptide MICs for the 1445 strains is displayed in Figure 1 according to the year of isolation and to gentamicin susceptibility. Overall, vancomycin MICs ranged from 0.5 to 6.0 mg/L (MIC_{50} 2.0 mg/L) for GR-MRSA and from 0.5 to 2.0 mg/L (MIC_{50} 1.5 mg/L) for GS-MRSA. Teicoplanin MICs ranged from 0.25 to 16.0 mg/L (MIC_{50} 2.0 mg/L) for GR-MRSA and from 0.25 to 3.0 mg/L (MIC_{50} 1.5 mg/L) for GS-MRSA. The correlation between vancomycin MICs and teicoplanin MICs was higher for GR-MRSA ($R = 0.76$) (Table 1) than for GS-MRSA ($R = 0.57$) (Table 2).

None of the GS-MRSA strains had a glycopeptide MIC >3 mg/L and all these strains were clearly susceptible to both vancomycin and teicoplanin. Only one GR-MRSA strain had a vancomycin MIC >4 mg/L (MIC 6 mg/L) and was therefore classified as vancomycin-intermediate *S. aureus* (VISA) according to CA-SFM or NCCLS breakpoints. In contrast, a total of 194 (18%) GR-MRSA strains (including the VISA strain) met the CA-SFM definition for teicoplanin-intermediate *S. aureus* (TISA, teicoplanin MIC >4 mg/L), and 31 of the latter met the

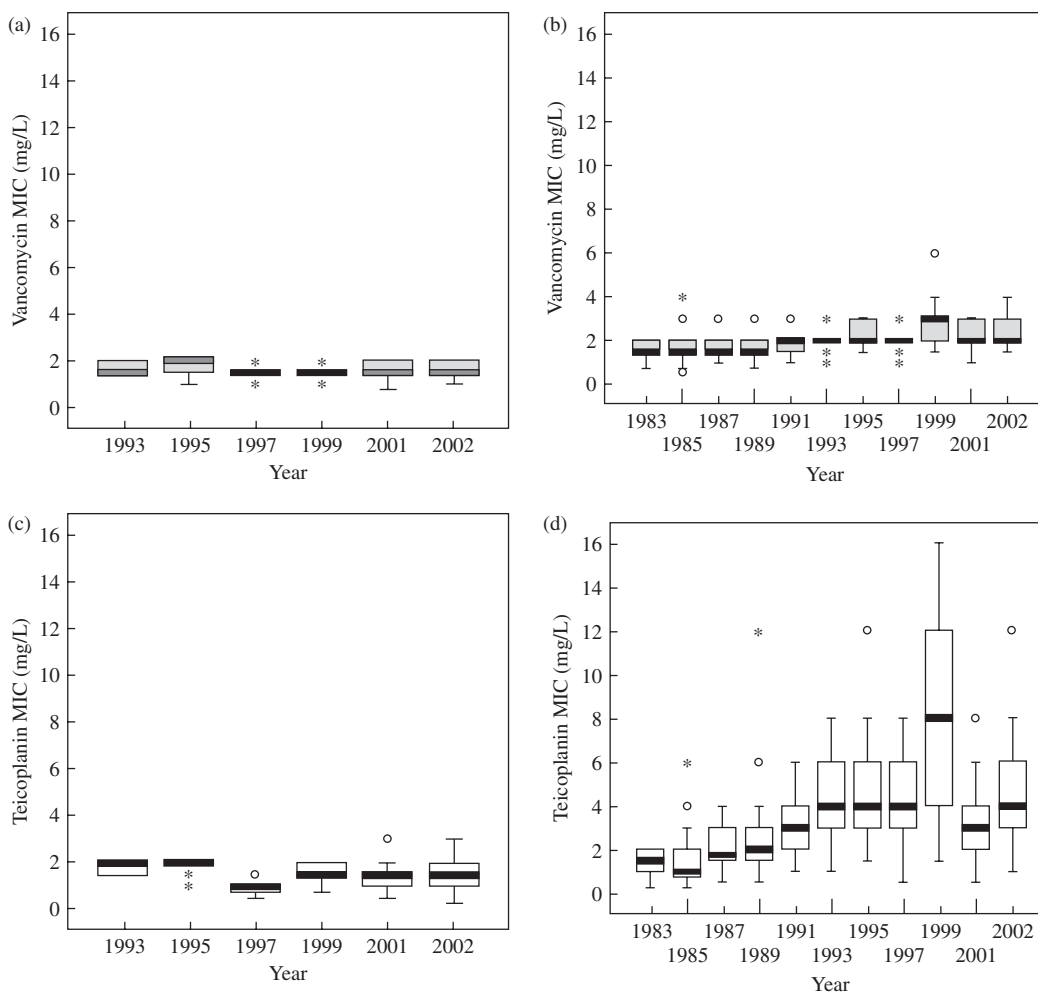


Figure 1. Box and whisker plots of glycopeptide MICs (i.e. vancomycin and teicoplanin MIC distributions over time) for GS-MRSA (a,c) and GR-MRSA strains (b,d). Median, bold horizontal bar. Outliers and extreme points are denoted by circles and asterisks.

Glycopeptide resistance in MRSA

Table 1. Correlation between vancomycin and teicoplanin MICs among GR-MRSA

No. of strains with a teico- planin MIC	No. of strains with a vancomycin MIC								Total strains
	0.50	0.75	1.00	1.50	2.00	3.00	4.00	6.00	
0.25			21	14	1				36
0.50	1	3	12	34	3				53
0.75	1	3	17	25	5				51
1.00		1	37	65	21				124
1.50			13	99	29				141
2.00			3	79	113	1			196
3.00			1	15	88	15			119
4.00				4	119	37	1		161
6.00				1	50	63	4		118
8.00					7	36	2		45
12.00					2	19	6	1	28
16.00						1	2		3
Total strains	2	7	104	336	438	172	15	1	1075

Table 2. Correlation between vancomycin and teicoplanin MICs among GS-MRSA

No. of strains with a teico- planin MIC	No. of strains with a vancomycin MIC								Total strains
	0.50	0.75	1.00	1.50	2.00	3.00	4.00	6.00	
0.25			1						1
0.50			12	2					14
0.75		1	4	14					19
1.00			11	69	8				88
1.50			8	83	33				124
2.00				47	72				119
3.00					5				5
Total strains		1	36	215	118				370

Table 3. Vancomycin and teicoplanin MICs of GR-MRSA

Year	No. of strains	Vancomycin				Teicoplanin			
		MIC ₅₀	MIC ₉₀	Range	G-mean	MIC ₅₀	MIC ₉₀	Range	G-mean
1983	172	1.50	2.00	(0.75–2.00)	1.56	1.50	2.00	(0.25–2.00)	1.10
1985	253	1.50	2.00	(0.50–4.00)	1.53	1.00	3.00	(0.25–6.00)	1.17
1987	40	1.50	2.00	(0.75–3.00)	1.66	1.75	4.00	(0.50–4.00)	1.80
1989	104	1.50	2.00	(0.75–3.00)	1.60	2.00	4.00	(0.50–12.00)	2.08
1991	62	2.00	3.00	(1.00–3.00)	1.90	3.00	6.00	(1.00–6.00)	2.76
1993	52	2.00	3.00	(1.00–3.00)	1.99	4.00	8.00	(1.00–8.00)	3.84
1995	87	2.00	3.00	(1.50–3.00)	2.16	4.00	8.00	(1.50–12.00)	3.77
1997	78	2.00	3.00	(1.00–3.00)	2.08	4.00	6.00	(0.50–8.00)	3.39
1999	69	3.00	4.00	(1.50–6.00)	2.61	6.00	12.00	(1.50–16.00)	6.31
2001	84	2.00	3.00	(1.00–3.00)	2.09	3.00	6.00	(0.50–12.00)	2.97
2002	74	2.00	3.00	(1.50–4.00)	2.41	4.00	6.00	(1.00–12.00)	4.31
Total	1075	2.00	3.00	(0.50–6.00)	1.82	2.00	6.00	(0.25–16.00)	2.16

G-mean, geometric mean.

NCCLS definition for TISA (teicoplanin MIC >8 mg/L). All but 2 of the 31 strains with a teicoplanin MIC >8 mg/L had a vancomycin MIC ≥3 mg/L.

Of note, while no time trend in glycopeptide MICs was observed for GS-MRSA, there was a significant upward trend over time in MICs of both vancomycin and teicoplanin for GR-MRSA (Figure 2). First, all but three of the 194 TISA strains were observed from 1989 onwards. Second, vancomycin MIC geometric mean increased from 1.56 mg/L in 1983 to 2.41 mg/L in 2002 ($P < 0.01$; t -test for the slope), and teicoplanin MIC geometric mean increased even more markedly from 1.10 mg/L in 1983 to 4.31 mg/L in 2002 ($P < 0.01$; t -test for the slope) (Table 3). This trend was equally observed when MIC₅₀ and MIC ranges were analysed (Figure 1).

Discussion

Clinical strains of GISA have been regularly reported from many parts of the world over the last 10 years.^{2–5} However, the real

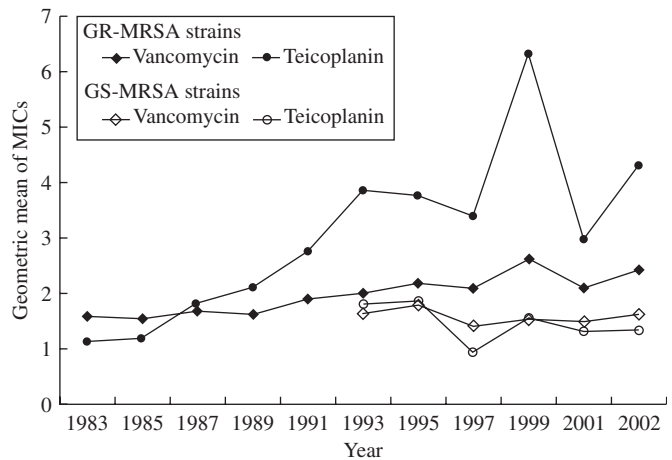


Figure 2. Evolution of the MIC geometric means of vancomycin and teicoplanin for GR-MRSA and GS-MRSA strains.

magnitude of this phenomenon remains to be delineated because of the difficulties to systematically perform MIC determination in most diagnostic laboratories. For that reason, specific surveys such as this one seem to be an accurate way to give a picture of this emergent issue. In this study, by using MIC determination by a standardized Etest method as recommended by NCCLS and CA-SFM, we were able to demonstrate that GISA strains emerged in our hospital in the late 1980s and became more frequent in the 1990s. Most of these strains are considered as TISA but remained susceptible to vancomycin, although they displayed increased MICs (≥ 3 mg/L) to the latter drug. In addition, there was a significant increase in MICs of both glycopeptides over the years. Of note, GISA strains and upward trends in glycopeptide MICs were observed only in GR-MRSA.

We report a significant increase in glycopeptide, and especially teicoplanin, geometric mean MIC over the last 20 years, although most GR-MRSA remained susceptible to glycopeptides when breakpoints were strictly applied. As found in other reports,⁷ GISA strains existed in our hospital in the late 1980s, confirming that this was not a new phenomenon when the first descriptions were published in 1995.² No strain with high level of glycopeptide resistance due to *vanA*, as described very recently in US hospitals, was observed in this study.¹⁴ There was also a good correlation between vancomycin and teicoplanin MICs among GR-MRSA strains. Consequently, increased teicoplanin MIC may indicate a first step towards VISA and GISA. It is not surprising that most GISA strains are less susceptible to teicoplanin than to vancomycin. Indeed, teicoplanin resistance has been reported prior to vancomycin resistance for both coagulase-negative staphylococci and *S. aureus*.^{2,15} This may reflect the lower baseline activity of teicoplanin on staphylococci as compared with vancomycin making the emergence of resistance more likely to appear with teicoplanin. Consequently, determining teicoplanin MIC of MRSA may be of major interest.

Of interest, in this study as in others, is that all strains with increased glycopeptide MICs were gentamicin resistant, although a few GS-MRSA strains have been reported as GISA around the world.^{4,12,16,17} Since GS-MRSA replaced GR-MRSA in most European hospitals, resulting in a decrease in the number of GR-MRSA isolated in clinical microbiology laboratories,¹⁸ it may be a cost-effective policy to search for GISA on GR-MRSA strains.

To date, no satisfactory routine method other than MIC could be used to screen for GISA. The disc diffusion method has been shown to be inadequate.⁸ The use of an agar screening test with brain–heart infusion or Mueller–Hinton media has proven to be very sensitive but poorly specific since up to 95% of strains that grow on such media may not be confirmed as GISA.^{12,19} As suggested by Tenover *et al.*,¹¹ it is advisable that hospitals develop an algorithm to screen for GISA because screening all MRSA strains with MIC determination may not be cost-effective. Indeed, overall, only 18% of GR-MRSA and less than 10% of all MRSA were GISA in the present study.

We did not search for hetero-GISA, because there is currently no standardized method to detect hetero-GISA and the clinical significance of such strains is debated.^{11–13} Consequently, we may have underestimated the proportion of MRSA strains with modified susceptibility to glycopeptides. We preferred to use a clear, although limited, definition of GISA based on standardized MIC determination. We did not perform genetic relatedness analysis of all MRSA strains and especially GISA strains. Therefore, clonal

spread cannot be definitely excluded, especially for the year 1999 where a peak of resistance could be observed. However, it has been previously shown that GISA strains are mainly related to well-described MRSA clones, and that in France most strains belong to a single genotype including both hetero-GISA and GISA strains.¹⁶

In conclusion, GISA have existed in our institution for more than 15 years, and there is a concerning increase in glycopeptide MICs for MRSA strains isolated. Such an increase warrants close surveillance because GISA epidemics may emerge. We suggest that, in countries where MRSA is a major health issue, GR-MRSA strains be systematically tested for teicoplanin susceptibility by MIC determination.

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Transparency declarations

None to declare.

References

1. Tiemersma EW, Bronzwaer SLAM, Lyytikäinen O *et al.* Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg Infect Dis* [serial on the Internet] 2004. <http://www.cdc.gov/ncidod/EID/vol10no9/04-0069.htm>. (6 September 2005, date last accessed).
2. Mainardi JL, Shlaes DM, Goering RV *et al.* Decreased teicoplanin susceptibility of methicillin-resistant strains of *Staphylococcus aureus*. *J Infect Dis* 1995; **171**: 1646–50.
3. Hiramatsu K, Hanaki H, Ino T *et al.* Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40**: 135–6.
4. Fridkin SK, Hageman J, McDougal LK *et al.* Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2001. *Clin Infect Dis* 2003; **36**: 429–39.
5. Rotun SS, McMath V, Schoonmaker DJ *et al.* *Staphylococcus aureus* with reduced susceptibility to vancomycin isolated from a patient with fatal bacteremia. *Emerg Infect Dis* 1999; **5**: 147–9.
6. Tabaqchali S. Vancomycin-resistant *Staphylococcus aureus*: apocalypse now? *Lancet* 1997; **350**: 1644–5.
7. Cercenado E. Glycopeptide-intermediate *Staphylococcus aureus*: rediscovery of an old problem? *Clin Microbiol Infect* 2000; **6**: 517–8.
8. Tenover FC, Lancaster MV, Hill BC *et al.* Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J Clin Microbiol* 1998; **36**: 1020–7.
9. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition. Susceptibility Tests for Bacteria That Grow Aerobically M02-A8*. NCCLS, Wayne, PA, USA.
10. Comité de l'Antibiogramme de la Société Française de Microbiologie. *Report* 2005. http://www.sfm.asso.fr/doc/download.php?doc=DiU8C&fic=Communiqué_2005.pdf. (6 September 2005, date last accessed).
11. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. *Emerg Infect Dis* 2001 [Online] Mar–Apr. <http://www.cdc.gov/ncidod/eid/vol7no2/tenover.htm> (6 September 2005, date last accessed).

Glycopeptide resistance in MRSA

12. Denis O, Nonhoff C, Byl B *et al.* Emergence of vancomycin-intermediate *Staphylococcus aureus* in a Belgian hospital: microbiological and clinical features. *J Antimicrob Chemother* 2002; **50**: 383–91.
13. Guerin F, Buu-Hoi A, Mainardi JL *et al.* Outbreak of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to glycopeptides in a Parisian hospital. *J Clin Microbiol* 2000; **38**: 2985–8.
14. Centers for Disease Control and Prevention (CDC). Vancomycin-resistant *Staphylococcus aureus*—Pennsylvania, 2002. *MMWR* 2002; **51**: 902.
15. Wilson AP, O'Hare MD, Felmingham D, Gruneberg RN. Teicoplanin-resistant coagulase-negative *staphylococcus*. *Lancet* 1986; **2**: 973.
16. El Solh N, Davi M, Morvan A *et al.* Characteristics of French methicillin-resistant *Staphylococcus aureus* isolates with decreased susceptibility or resistance to glycopeptides. *J Antimicrob Chemother* 2003; **52**: 691–4.
17. Howe RA, Monk A, Wootton M *et al.* Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. *Emerg Infect Dis*. [Online] 2004 May. <http://www.cdc.gov/ncidod/EID/vol10no5/03-0556.htm> (6 September 2005, date last accessed).
18. Jarlier V. Bactéries multirésistantes dans les hôpitaux français: des premiers indicateurs au Réseau d'alerte d'investigation et de surveillance des infections nosocomiales (Raisin). *Bull Epid Hebdo* [serial on the Internet] 2004; **32–33**: 148–151. http://www.invs.sante.fr/beh/2004/32_33/beh_32_33_2004.pdf (6 September 2005, date last accessed).
19. Hubert SK, Mohammed JM, Fridkin SK *et al.* Glycopeptide-intermediate *Staphylococcus aureus*: evaluation of a novel screening method and results of a survey of selected U.S. hospitals. *J Clin Microbiol* 1999; **37**: 3590–3.