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Outbreak of Methicillin-Resistant *Staphylococcus aureus* with Reduced Susceptibility to Glycopeptides in a Parisian Hospital

FRANÇOIS GUERIN,[1] ANNIE BUU-HOÏ,[1] JEAN-LUC MAINARDI,[1,2] GUILLAUME KAC,[1] NATHALIE COLARDELLE,[1] SABINE VAUPRÉ,[1] LAURENT GUTMANN,[1,2] AND ISABELLE PODGLAJEN[1,2]*

Laboratoire de Microbiologie Médicale, Hôpital Broussais,[1] and Laboratoire de Recherche Moléculaire sur les Antibiotiques, UFR Broussais-Hôtel Dieu et Pitié-Salpétrière,[2] Université Paris VI, Paris, France

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Epidemiological relationships were investigated between 40 methicillin-resistant *Staphylococcus aureus* (MRSA) strains with decreased glycopeptide susceptibility isolated from November 1998 to March 1999 from 39 patients (17 infected and 22 colonized patients) in nine wards of the Broussais Hospital, Paris, France. Reduced glycopeptide susceptibility was readily detected on brain heart infusion (BHI) agar containing 6 μg of teicoplanin per ml and on gradient plates, but not by the standard disk diffusion method. The MICs of vancomycin and teicoplanin, determined on BHI agar, were 4 and 8 to 32 μg/ml, respectively (standard antibiotic dilution), and 4 to 8 and 8 to 32 μg/ml, respectively (E-test). All strains were resistant to macrolides, aminoglycosides, tetracycline, rifampin, sulfonamides, and pefloxacin, showed reduced susceptibility to fusidic acid and fosfomycin, and were susceptible to trimethoprim and chloramphenicol. Pulsed-field gel electrophoresis and lysotyping revealed that a multidrug-resistant MRSA clone with decreased susceptibility to glycopeptides has been discretely endemic since at least 1996 in our institution, where it was responsible for an outbreak in November and December 1998.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most important pathogens responsible for nosocomial infections. The glycopeptides vancomycin (VAN) and teicoplanin (TEC) are the drugs of choice for the treatment of infections due to MRSA. The MICs of these drugs for MRSA are generally 2 μg/ml or less (14). While reduced susceptibility to TEC (MICs, 8 to 16 μg/ml) in isolates of *S. aureus* has been reported since 1990 (2, 8, 9), clinical isolates of *S. aureus* with reduced susceptibility to VAN (MICs, 8 μg/ml) have occasionally been observed since 1996 (6, 11, 12). In these strains, reduced susceptibility to VAN is frequently associated with reduced susceptibility to TEC, which is then somewhat easier to detect (6, 11). To our knowledge, the dissemination of MRSA strains with reduced glycopeptide susceptibility has been documented only rarely, possibly due to the lack of ease of their detection. However, a high rate of occurrence of such strains was reported in university hospitals throughout Japan (7) and in one university hospital in Spain (1). Here, we describe an outbreak caused by an MRSA strain with reduced glycopeptide susceptibility which has been cryptically endemic in our hospital for several years and which is also present in other Parisian hospitals.

MATERIALS AND METHODS

Patients. From October 1998 through April 1999, 39 patients (13 women, 26 men) from nine wards of Broussais Hospital, predominantly from two intensive care units and one cardiology unit, were infected ($n = 17$) or colonized ($n = 22$) with MRSA strains with decreased susceptibility to glycopeptides (see Table 1).

Strains. All *S. aureus* strains studied except reference strain ATCC 25923 and strain 46063 were isolated at Broussais Hospital. We analyzed a total of 58 strains (see Table 1): 2 were susceptible to oxacillin and glycopeptides, 10 were glycopeptide-susceptible MRSA strains isolated during the study period, and the remaining 46 strains were homogeneously resistant MRSA and showed decreased susceptibility to glycopeptides. Among the last group of 46 strains, 40 were isolated during the 6-month study period, 5 were isolated either in 1996 or 1997, and 1 (strain 46063) was isolated in 1992 at Saint Joseph Hospital, Paris (9).

Antibiotics. Antibiotic disks were from Sanofi Diagnostics Pasteur (Marnes-La Coquette, France). Standard reference powders of VAN and TEC were obtained from Lilly (Saint-Cloud, France) and Marion Merrell Dow (Levallois-Perret, France), respectively.

Antimicrobial susceptibility testing. Isolates were tested by the disk diffusion method on Mueller-Hinton (MH) agar (bioMérieux, Marcy l'Etoile, France) by following the zone size criteria described by the Antibiogram Committee of the French Society of Microbiology (5). The antibiotics tested included penicillin G (PEN), oxacillin (OXA), rifampin (RIF), erythromycin (ERY), lincomycin (LIN), tetracycline (TET), streptomycin (STR), kanamycin (KAN), gentamicin (GEN), amikacin (AMK), tobramycin (TOB), sulfonamides (SUL), trimethoprim (TMP), chloramphenicol (CHL), fusidic acid (FUA), pefloxacin (PEF), fosfomycin (FOF), VAN, and TEC. The MICs of VAN and TEC were determined on MH and brain heart infusion (BHI) agar (Difco, Detroit, Mich.) with a Steers replicator device with an inoculum of ca. 10^4 CFU/spot and on BHI agar by the E-test (AB Biodisk, Dammartin sur Tigeaux, France) with an inoculum equivalent to 2 standard McFarland units. Each test was carried out at least three times. In the framework of this study, an isolate was considered to have decreased susceptibility if the MICs of VAN and TEC for the isolate were ≥4 μg/ml (3). Susceptibility to glycopeptides was also determined with BHI agar plates with VAN and TEC gradients from 0 to 16 μg/ml and by population analysis on BHI agar as described previously (7, 12).

PFGE. Pulsed-field gel electrophoresis (PFGE) was carried out with a CHEF DRII apparatus (Bio-Rad Laboratories, Ivry-sur-Seine, France) as described previously (13). *Sma*I- and *Sac*II-digested DNA was separated for 24 h at 14°C and 200 V with pulse times of 5 to 35 and 2 to 20 s and at an angle of 120°.

Lysotyping. The strains were phage typed at the National Reference Center for Staphylococci, Institut Pasteur, with the international set of *S. aureus* phages together with some experimental phages.

RESULTS

Analysis of the antimicrobial susceptibilities of the MRSA strains with decreased susceptibility to glycopeptides. The resistance markers for the strains studied are listed in Table 1. All 46 strains with decreased susceptibility to glycopeptides showed a particular multidrug resistance phenotype: resistance to PEN, OXA, RIF, ERY, LIN, TET, STR, KAN, GEN, AMK, TOB, SUL, and PEF and decreased susceptibility to FOF and FUA. The strains remained susceptible only to TMP and CHL. On the standard antibiogram, they yielded inhibition zones around the VAN and TEC disks with diameters of ≥17

* Corresponding author. Mailing address: L.R.M.A., Université Paris VI, 15 rue de l'Ecole de Médecine, 75270 Paris Cedex 06, France. Phone: 33-1-01.42.34.68.65. Fax: 33-1-01.43.25.68.12. E-mail: ipodgla@ccr.jussieu.fr.

TABLE 1. Characteristics of *S. aureus* strains analyzed

Strain[a]	Date of isolation (mo/yr)	Origin (infection [I] or colonization [C])	PFGE pattern	Phage type[b]	MIC (μg/ml)			
					VAN		TEC	
					Agar dilution method with MH agar/BHI agar	E-test	Agar dilution method with MH agar/BHI agar	E-test
Glycopeptide-susceptible strains								
ATCC 25923[c]			B	ND[d]	2/2	2	2/2	2
RA48[c]	01/99	Blood (I)	C	III	1/1	2	1/2	2
DE49[e]	08/98	Wound (C)	D	NT[f]	1/2	3	1/2	3
HE50[e]	11/98	PBS[g] (I)	E	ND	1/1	2	1/2	2
HO51[e]	12/98	Urine (I)	E (2)[h]	ND	1/2	3	1/2	2
MB52[e]	01/99	Wound (I)	F	IV	1/1	2	1/1	2
LO53[e]	01/99	Wound (C)	G	V	1/1	2	1/1	2
SE54[e]	01/99	Wound (C)	ND	NT	2/2	2	1/2	2
BA55[e]	01/99	Wound (C)	H	ND	1/1	2	1/2	2
ST56[e]	02/99	Urine (I)	H	ND	ND	3	ND	2
CR57[e]	03/99	PBS (I)	E	ND	ND	2	ND	1.5
CH58[e]	03/99	PBS (I)	E	VI	ND	2	ND	2
Strains with decreased susceptibility to glycopeptides[e]								
46063	11/92	Blood (I)	A	II	2/4	4	8/8	12
PE41	10/96	Blood (I)	A	I	4/4	6	4/8	12
KE42	06/97	Wound (C)	A	I	4/4	4	4/8	8
TR43	07/97	PBS (I)	A (1)	I	4/4	4	4/8	12
BE44	08/97	Wound (C)	A	I	4/4	6	4/8	16
RI45	09/97	Wound (C)	A	I	4/4	4	4/8	8
MO01	10/98	Blood (I)	A	I	2/4	4	4/8	12
CA02	10/98	Blood (I)	A	I	4/4	4	4/8	8
PE03	11/98	Drainage fluid (I)	A	I	2/4	4	4/8	12
ER04	11/98	Sputum (I)	A	I	4/4	4	4/8	12
MA05	11/98	PBS (I)	A	NT	2/4	4	4/8	12
BE06	11/98	Wound (I)	A	I	4/4	6	4/8	12
LA07	11/98	Wound (C)	A	I	4/4	4	4/8	12
DA08	11/98	Wound (C)	A	I	4/4	6	4/8	12
BO09	11/98	Nose (C)	A	I	4/4	6	4/8	12
GN10	11/98	PBS (C)	A	I	4/4	6	4/8	12
HA11	11/98	Wound (C)	A	NT	4/4	4	4/4	8
HE12	11/98	PBS (I)	A	I	4/4	4	4/8	16
SM13	12/98	Wound (C)	A	I	4/4	4	4/8	12
RI14	12/98	PBS (C)	A	NT	4/4	4	4/8	12
BE15	12/98	Catheter (I)	A	I	4/4	4	4/8	12
ZE16	12/98	Nose (C)	A	I	4/4	4	4/8	16
DE17	12/98	PBS (I)	A	I	4/4	6	4/8	12
DE18	12/98	Wound (I)	A (4)	I	4/4	8	16/32	32
TE19	12/98	Wound (C)	A	I	4/4	4	4/8	12
SI20	12/98	Catheter (I)	A	I	4/4	6	4/8	12
RA21	12/98	Wound (C)	A	I	4/4	4	4/8	12
GA22	12/98	Sputum (C)	A	I	4/4	6	4/8	12
LA23	01/99	Catheter (C)	A	I	4/4	6	4/8	12
TU24	01/99	PBS (C)	A	NT	4/4	4	4/4	12
HE25	01/99	Electrode (I)	A	I	4/4	6	4/8	12
PI26	01/99	PBS (I)	A	NT	4/4	6	4/8	12
FA27	01/99	Wound (C)	A	I	4/4	4	4/8	12
LE28	01/99	Wound (C)	A	I	4/4	6	4/8	16
ES29	01/99	Sputum (C)	A	I	4/4	6	4/8	16
VE30	02/99	Wound (C)	A	NT	4/4	4	4/8	12
PO31	02/99	Wound (C)	A	I	4/4	4	4/8	12
AU32	02/99	Urine (I)	A	I	4/4	4	4/8	12
OB33	02/99	Urine (I)	A	I	4/4	4	4/8	12
KA34	02/99	Pleural catheter (C)	A	ND	4/4	4	4/8	12
JO35	02/99	Catheter (I)	A	ND	4/4	6	4/8	16
LA36	02/99	Nose (C)	A (4)	ND	4/4	8	4/8	16
GA37	03/99	Blood (I)	A	ND	4/4	6	4/8	16
CA38	03/99	Wound (I)	A	ND	4/4	6	4/8	12
HA39	03/99	Nose (C)	A (1)	ND	4/4	4	4/8	8
TE40	03/99	Rectal (C)	A	ND	4/4	4	4/8	12

[a] All strains except ATCC 25923 and strain 46063 were isolated at Broussais Hospital. Strain ATCC 25923 was from the American Type Culture Collection, and strain 46063 was isolated at Saint Joseph Hospital (9).
[b] Phage type designations were arbitrarily assigned (I through VI).
[c] Methicillin susceptible.
[d] ND, not determined.
[e] Methicillin resistant.
[f] NT, not typeable.
[g] PBS, protected brush specimen.
[h] The values in parentheses are the number of bands that differ with respect to the predominant pattern.

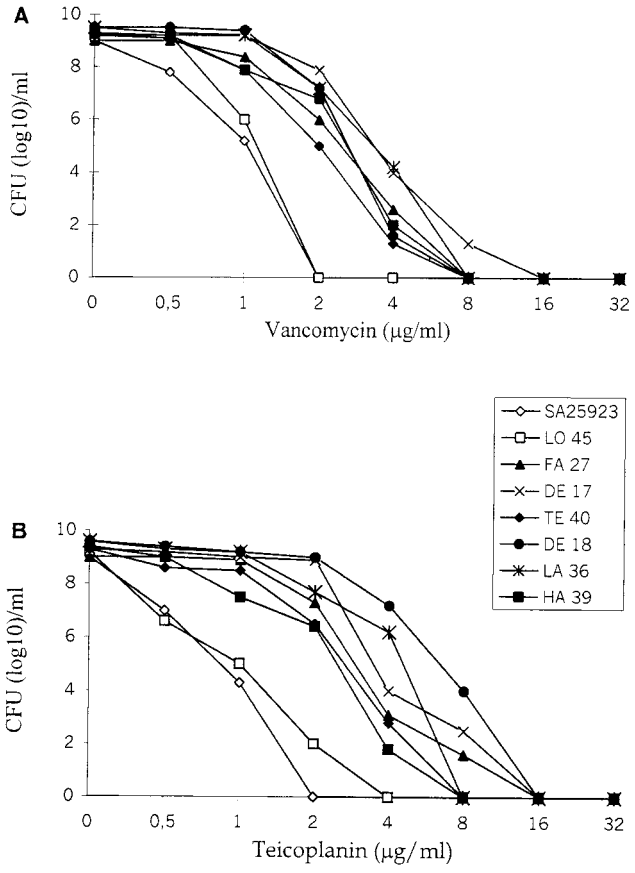

FIG. 1. Population analysis of *S. aureus* strains with decreased susceptibility to glycopeptides (strains FA27, DE17, TE40, DE18, LA36, and HA39). (A) Analysis carried out on plates with VAN; (B) analysis carried out on plates with TEC. Strains SA25923 and LO45 were fully glycopeptide susceptible.

mm; however, two strains (BE06 and DE18) had diameters of ca. 15 mm around the TEC disks. With respect to the MICs for the 12 fully susceptible strains including the American Type Culture Collection (ATCC) reference strain (Table 1), the MICs of VAN for the 46 strains under study were increased twofold on BHI agar, while the MICs of TEC were increased 4- to 16-fold. When the E-test was used, similar increases were observed and reduced susceptibilities to both glycopeptides were obvious when the multidrug-resistant MRSA strains were grown on gradient plates (data not shown). Ninety-seven percent of these strains grew on BHI agar containing 6 μg of TEC per ml.

Figure 1 shows a comparative population analysis of six MRSA strains with reduced susceptibility to glycopeptides and two glycopeptide-susceptible strains. The two categories were clearly distinct. MRSA strains with reduced glycopeptide susceptibility contained subpopulations able to grow on 4 μg of VAN per ml at frequencies of 10^{-7} to 10^{-5}, while the susceptible strains were fully inhibited by 2 μg/ml (Fig. 1A). Further population analysis of the subpopulations that grew in the presence of VAN at a concentration of 4 μg/ml showed that they remained heterogeneous (data not shown). Similar results were obtained with TEC, but the frequencies were somewhat higher (Fig. 1B). These strains might then be called hetero-VAN-resistant *S. aureus* (hetero-VRSA), as proposed by Hiramatsu et al. (7), or hetero-glycopeptide-resistant *S. aureus* (hetero-GRSA).

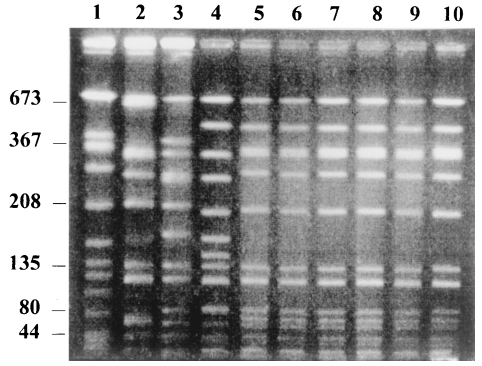

FIG. 2. PFGE profiles of *S. aureus* strains. Lanes: 1, MRSA1152 (Spanish isolate [1]); 2, Mu50 [Japanese isolate [6]); 3, fragment size marker (in kilobase pairs; indicated on the left margin); 4, MB52; 5, CA38; 6, OB33; 7, SI20; 8, CA02; 9, PE 41; 10, MRSA 46063. All strains except MB52 had reduced susceptibility to glycopeptides.

Epidemiological markers of the MRSA strains with decreased susceptibility to glycopeptides. Table 1 summarizes the results of the analysis of the PFGE banding patterns and the lysotypes of all strains included in this study. Ninety-three percent of the 40 strains with reduced glycopeptide susceptibility isolated during the 6-month study period had identical patterns. The remaining 7% of the strains had banding patterns similar to the predominant pattern, with differences concerning no more than four bands and indicating a close relationship between the strains (13). Five strains isolated at Broussais Hospital in 1996 and 1997, and one strain, 46063, isolated in 1992 at Saint Joseph Hospital (9) yielded the predominant banding pattern (Fig. 2). By contrast, differences in at least seven bands were consistently observed for the fully glycopeptide-susceptible MRSA strains (Fig. 2). As for the phage types, all typeable strains with reduced susceptibility to glycopeptides except strain 46063 had identical lysotypes and were susceptible only to phage 54, a lysotype uncommon in fully glycopeptide-susceptible strains in France (N. El Solh, personal communication).

DISCUSSION

In our institution, a 400-bed university hospital, about 1,000 *S. aureus* strains are isolated annually. In 1998, 40% of the isolates were MRSA, and about one-half of these were susceptible to GEN. The observation, at an unusual frequency, of MRSA strains with a particular phenotype (resistance to GEN, RIF, ERY, LIN, TET, PEF, and SUL, reduced susceptibility to FUA, and susceptibility to TMP and CHL) in November 1998 led us to analyze these strains more closely. Forty-five MRSA strains with the particular multiple-antibiotic resistance phenotype were collected retrospectively and prospectively from October 1998 until the end of March 1999.

The glycopeptide susceptibilities of these multidrug-resistant strains were not adequately assessed by the standard antibiogram procedure, since only in some rare instances was there a marginally reduced diameter of the inhibition zone around TEC. Considering the only slightly reduced susceptibilities observed after MIC determinations by standard methods, these strains do not fall into the category of intermediate glycopeptide susceptibility according to the criteria of the National Committee for Clinical Laboratory Standards (10) and the Antibiogram Committee of the French Society of Microbiology (5). They were, however, not fully glycopeptide susceptible as were the MRSA strains with different multiple-antibi-

otic resistance profiles isolated in our institution during the same period. When the standard dilution procedure was carried out on BHI medium instead of MH medium, as proposed previously (7), reduced susceptibility to TEC became noticeable (Table 1). This was also the case when gradient plates or the E-test was used with BHI medium (Table 1).

Reduced glycopeptide susceptibility was not a homogeneously expressed trait. The subpopulation that grew in the presence of more than 4 μg of VAN or TEC per ml among the strains with reduced glycopeptide susceptibility (Fig. 1) was reminiscent of the previously reported subpopulation strains termed hetero-VRSA (7, 12). It would therefore be reasonable to consider the presence of such strains a possible risk factor for VAN or TEC treatment failures in patients with MRSA infections since this reduction in susceptibility might be the first step in the development of higher-level resistance.

Molecular typing and lysotyping revealed a high degree of relatedness among the strains with reduced glycopeptide susceptibility. This, in conjunction with the fact that the lysotype of these isolates was rare and the fact that the multidrug resistance phenotype was homogeneous, suggested that these strains were clonal. The identification of strains of this type among hospital strains dating back to 1996, the increase in their frequency over a 2-month period, and the persistence of scattered isolates to the present day, despite drastic measures related to hospital hygiene, are consistent with a limited outbreak of a discretely endemic strain. This persistence probably dates back to at least 1992, when MRSA strain 46063 (which has the clonal PFGE pattern; Fig. 2) was isolated in a hospital immediately adjacent to Broussais Hospital (9). When the PFGE patterns of the MRSA isolates with reduced glycopeptide susceptibility from our institution were compared with that of strain Mu50, which is related to the hetero-VRSA strains from Japan (7), it was obvious that they were not members of the same clone (Fig. 2). The same observation was made (Fig. 2) with respect to hetero-VRSA strains from Spain (1).

Four of the 40 consecutive strains isolated during the 6-month study period were isolated from patients upon admission. This indicated that the clonal isolates are not confined to our institution. In fact, MRSA isolates with indistinguishable characteristics have since been identified in at least three major hospitals in Paris (data not shown). We do not know the frequencies at which MRSA with reduced glycopeptide susceptibility occur in those institutions and whether they have constituted up to 25% of all staphylococci isolated during any given period, as was the case during the last 2 months of 1998 in Broussais Hospital.

Prospectively, all MRSA isolates should be systematically examined for reduced glycopeptide susceptibility because it may not remain confined to the multiple antibiotic-resistant clones identified so far. Clonal diversity of hetero-VRSA strains isolated in different French hospitals was recently demonstrated unambiguously (4). In the absence of broadly recognized recommendations, we now routinely screen all *S. aureus* strains on BHI agar containing 6 μg of TEC per ml. If reduced glycopeptide susceptibility is suspected, it is verified by the E-test with VAN and TEC. However, all *S. aureus* strains isolated from patients with severe infections are immediately subjected to the E-test.

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