ELSEVIER

Journal of Cystic Fibrosis 10 (2011) 377 – 382

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Case Studies

Staphylococcus aureus with decreased susceptibility to glycopeptides in cystic fibrosis patients ☆

Anne Filleron [a], Raphaël Chiron [b], Marie-Elisabeth Reverdy [c], Hélène Jean-Pierre [d,e], Oana Dumitrescu [c], Linda Aleyrangues [d], François Counil [b], Estelle Jumas-Bilak [e], Hélène Marchandin [d,e,*]

[a] *Centre Hospitalier Régional Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Service de Pédiatrie générale, Maladies Infectieuses, Immunologie Clinique, 371, Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France*

[b] *Centre Hospitalier Régional Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Centre de Ressources et de Compétences pour la Mucoviscidose, Service de Pédiatrie 1 et des Maladies Respiratoires, 371, Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France*

[c] *Centre National de Référence des Staphylocoques, INSERM E0230, Faculté de Médecine Laennec, 7 rue Guillaume Paradin, 69008 Lyon, France*

[d] *Centre Hospitalier Régional Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Bactériologie, 371, Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France*

[e] *Université Montpellier 1, UMR 5119 (UM2, CNRS, IRD, IFREMER, UM1), Equipe Pathogènes et Environnements, U.F.R. Pharmacie, 15, Avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France*

Received 13 February 2011; received in revised form 23 April 2011; accepted 1 May 2011
Available online 1 June 2011

Abstract

We report the isolation of *Staphylococcus aureus* with decreased susceptibility to glycopeptides in five CF patients and review the clinical and microbiological features of these cases. Three patients presented with pulmonary exacerbation that may be attributed to these strains and two of them were successfully treated using linezolid therapy. Glycopeptide-intermediate *S. aureus* (GISA) strains isolated in two patients were susceptible to methicillin, while the three other patients harbored methicillin-resistant GISA. Rarely reported in CF, GISA may remain underestimated due to the difficulty of detection, and both clinicians and microbiologists should be aware of the GISA emergence in CF patients' population.

Keywords: Glycopeptides; Resistance; *Staphylococcus aureus*; GISA; Cystic fibrosis

1. Introduction

Glycopeptide-intermediate *Staphylococcus aureus* (GISA) was first reported in a CF patient in 2002. Since then, 12 additional isolates showing heterogeneous resistance (heterogeneous GISA, hGISA) were reported in CF patients, suggesting that *S. aureus* with decreased susceptibility to glycopeptides could be emergent in the CF population [2-4]. Clinical implication of these strains was documented once, the first reported strain being involved in bronchopulmonary exacerbation [2-4]. We report here clinical and microbiological features of GISA/hGISA isolation in five CF patients with the aim to contribute to knowledge on these as-yet-rarely isolated strains in CF and their implication in the respiratory status of patients. Eight GISA/hGISA strains were isolated from these five patients attending the CF center of the Montpellier University Hospital, a large regional CF center caring for about 200 children and adults each year (95 adults and 110 children in 2009).

☆ This study was presented in part at the 32nd European Cystic Fibrosis Society Conference, Brest, France, 10–13 June 2009 [1].

* Corresponding author at: UMR 5119, Equipe Pathogènes et Environnements, U.F.R. Pharmacie, 15, avenue Charles Flahault BP 14491, 34093 Montpellier Cedex 5, France. Tel.: +33 4 67 63 54 26; fax: +33 4 67 63 45 11.
E-mail address: helene.marchandin@univ-montp1.fr (H. Marchandin).

1569-1993/$ - see front matter © 2011 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2011.05.001

2. Case 1, a 17-year-old patient with 2-year chronic colonization by a GISA/MRSA strain

In 2004, the 12-year-old patient was first colonized by methicillin-resistant *S. aureus* (MRSA) and his clinical and respiratory status rapidly worsened (body mass index (BMI) 11 kg/m^2; forced expiratory volume in 1 s (FEV_1), 37% pred) requiring gastrostomy, continuous oxygen therapy and multiple courses of IV antibiotics, of which eight included teicoplanin (12 to 26 mg/kg/day for 48 h followed by 6 mg/kg/day for 14 to 21 days with monitoring of blood concentration). However, an MRSA was still isolated from multiple samples until the end of 2004. In 2005, four courses of antimicrobial therapy with linezolid for 15 days led to clinical improvement with a FEV_1 increasing by 220 ml and no MRSA isolated from several subsequent samples. A lung transplant was performed in October 2005 (FEV_1 44% pred) and the immediate post-transplant clinical evolution was favorable (FEV_1 76% pred, January 2006). In the absence of sputum production, no bacteriological analysis was performed until March 2006. In March 2006, a GISA/MRSA strain was the only pathogen cultured from a sputum sample (Table 1). The antimicrobial susceptibility pattern was identical to that of strains isolated in 2004. Since the clinical status of the patient remained stable, no treatment against MRSA was prescribed until 2007. In 2007, a 10% progressive decline in FEV_1 was noted and the patient received three courses of anti-MRSA treatment including pristinamycin and/or trimethoprim-sulfamethoxazole and/or rifampin despite the absence of pulmonary exacerbation. However, several MRSA strains with identical antibiotypes were isolated until March 2008. Among them, two strains isolated in May 2007 and March 2008 were identified as GISA (Table 1). In June 2008, graft chronic rejection occurred but no infectious signs were noted. The patient died from respiratory failure in August 2009. In this case, two-year GISA chronic colonization (from March 2006 to March 2008) has been documented on the basis of identical genotypic characteristics of the three confirmed GISA isolates but the clinical implication remains undetermined.

3. Case 2, a 24-year-old patient with successive isolation of two unrelated GISA/MSSA responsible for pulmonary exacerbation successfully treated by linezolid

In 2004, the clinical condition of patient 2, a 20-year-old, worsened (BMI 17 kg/m^2; FEV_1 63% pred, moderate bronchiectasis). Colonization by a methicillin-susceptible (MSSA) strain was noted. The patient received four courses of antistaphylococcal treatment per year, which did not include glycopeptide but a combination of pristinamycin and/or rifampin and/or amoxicillin-clavulanic acid, but no eradication of the strain was observed. In 2007, *Pseudomonas aeruginosa* chronic colonization required antimicrobial cure every 3 months according to the antibiogram. In 2008, the patient experienced several episodes of pulmonary exacerbation, his clinical status worsened and monthly IV treatment alternatively against *S. aureus* (co-amoxiclav, 1 g three times daily for 15 days) and *P. aeruginosa* (ciprofloxacin or ceftazidime associated with tobramycin) was introduced in combination with nebulized tobramycin. The first GISA/MSSA strain was recognized during pulmonary exacerbation (FEV_1 48% pred) in August 2008. Retrospective analysis found an overlooked GISA/MSSA strain with distinct antibiotype isolated during an episode of pulmonary exacerbation (FEV_1 33% pred) in April 2008 (Table 1). Comparison of the genotypic characteristics revealed the two GISA strains to be unrelated. In October 2008, when GISA was confirmed, a linezolid course (600 mg b.i.d. for 15 days) led to clinical and respiratory function improvement. The patient remains clinically stable and no *S. aureus* strains were isolated from sputum samples for 1 year.

4. Case 3, a 19-year-old patient with pulmonary exacerbation and teicoplanin treatment failure attributed to GISA/MSSA and clinical improvement after linezolid therapy

Initial clinical decline was noted at the age of 16 years in 2004 (BMI 18 kg/m^2, FEV_1 78% pred, Bhalla score 10/25) despite IV antimicrobial therapy against *P. aeruginosa*. Chronic colonization by MRSA had been noted since May 2005 and colonization by MSSA had been observed since March 2006. In 2006, regular treatment was started with an antimicrobial regimen given every 3 months. The patient received teicoplanin (400 mg daily for 2 days followed by 200 mg daily for 15 days) in August 2007, plus pristinamycin and rifampin for 3 weeks in September 2007. In November 2007, MSSA grown from sputum culture during pulmonary exacerbation showed decreased susceptibility to glycopeptides (Table 1). From January 2008, three courses of linezolid were administered (600 mg b.i.d. for 15 days every 3 months) due to treatment failure observed after 15-day teicoplanin treatment, leading to clinical improvement. The clinical status remained stable throughout 2008 and no *S. aureus* were isolated during an 8-month period.

5. Case 4, a 37-year-old patient with pulmonary exacerbation attributed to GISA/MRSA after teicoplanin treatment

Colonization by MRSA occurred for 5 years (2004) in addition to MSSA, *P. aeruginosa* and *Aspergillus fumigatus* frequent recovery in the sputum samples. FEV_1 was relatively stable until 2007 (between 60 and 70% pred). In 2008, anti-MRSA antibiotic treatment was added to antimicrobial treatment against *P. aeruginosa* with linezolid in May 2008 (600 mg b.i.d. for 15 days) and teicoplanin in December 2008, October 2009 and November 2009 (400 mg daily for 15 days with monitoring of blood concentration) because of multiple pulmonary exacerbations and constant recovery of MRSA from sputum samples. A GISA/MRSA strain was isolated in November 2009 after 17 days of a combination of IV ceftazidime, tobramycin and teicoplanin given for the treatment of pulmonary exacerbation (FEV_1 43% pred) (Table 1). The patient refused any additional intravenous antimicrobial therapy. Oral combination of fusidic acid and minocycline was started for 15 days despite resistance to both drugs observed on the antibiogram, in combination with nebulized colimycin. Clinical status improved, probably due to associated aerosol therapy, but MRSA was not eradicated.

Table 1
Clinical and microbiological features of the cases of GISA/hGISA isolation in CF patients in this study [a].

Characteristic	Patient							
	Patient 1			Patient 2		Patient 3	Patient 4	Patient 5
Patient								
Sex	F			M		M	F	F
CF genotype	F508del/N1303K			F508del/F508del		F508del/F508del	G542X/R792X	F508del/F508del
Age at first *S. aureus* colonization	12 y			Unknown (<20 y)		Unknown (<15 y)	Unknown (<29 y)	7 y
Age at first MRSA colonization	12 y			No MRSA		Unknown (<15 y)	31 y	7 y
Age at hGISA/GISA isolation	14 y			24 y		17 y	36 y	12 y
Therapeutic requirements within the 6 months preceding the isolation of GISA (excluding nebulized agents)	Teicoplanin, Levofloxacin, Ceftazidime, Linezolid, Azithromycin, Amoxicillin-clavulanic acid, Pristinamycin, Rifampin, Trimethoprim-sulfamethoxazole			Ciprofloxacin, Tobramycin, Ceftazidime, Amoxicillin-clavulanic acid		Teicoplanin, Ciprofloxacin, Tobramycin, Ceftazidime, Pristinamycin, Rifampin, Ticarcillin-clavulanic acid	Teicoplanin, Ciprofloxacin, Levofloxacin, Tobramycin, Ceftazidime, Trimethoprim-sulfamethoxazole	Ciprofloxacin, Tobramycin, Ticarcillin-clavulanic acid
GISA/hGISA isolation [b]								
Date	March 2006	May 2007	March 2008	April 2008	August 2008	November 2007	November 2009	February 2010
Sample	Sputum	Sputum	Sputum	BAL	Sputum	Sputum	Sputum	Sputum
Clinical status at GISA/hGISA recovery [c]	Stable	Unstable (Pulmonary stenosis)	Unstable (Chronic rejection)	Unstable	Unstable	Unstable	Unstable	Stable
Associated pathogens	None	GSSA/MRSA *Haemophilus influenzae*	*Candida albicans*	Mucoid *P. aeruginosa*	*P. aeruginosa* (mucoid and non-mucoid)	MRSA *P. aeruginosa* (mucoid and non-mucoid)	*P. aeruginosa*	GSSA/MRSA *P. aeruginosa* (mucoid and non-mucoid)
Methicillin-resistance phenotype	MRSA	MRSA	MRSA	MSSA	MSSA	MSSA	MRSA	MRSA
Initial detection mode [d]	Antibiotype	Systematic testing	Systematic testing	Retrospective testing	Antibiotype	Antibiotype	Treatment failure	Antibiotype
Vancomycin Etest MIC (mg/L)	4	2	3	2	2	3	2	1
Teicoplanin Etest MIC (mg/L)	8	2	3	2	6	3	3	6
Susceptibility/resistance to gentamicin	R	R	R	S	S	R	S	S
Susceptibility/resistance to rifampin	R	R	R	R	R	I	R	I
Resistance to other antimicrobial agents [e]	KAN, TOB, ERY, LIN, TET, OFX	KAN, TOB, ERY, LIN, TET, OFX	KAN, TOB, ERY, LIN, TET, OFX	KAN, TOB, ERY	ERY, LIN, FOF	KAN, TOB, ERY, LIN, TET, OFX, TMP	KAN, TOB, ERY, LIN, TET, OFX	KAN, TOB, ERY
Antimicrobial treatment	No	No	No	Piperacillin-tazobactam, Tobramycin	Ceftazidime, Tobramycin, Linezolid	Ciprofloxacin, Linezolid	Fusidic acid, Minocycline Colistin (aerosol)	Trimethoprim-sulfamethoxazole
Outcome	Stable	Unstable	Death August 2009	Unstable	Stable	Stable	Improvement	Stable

[a] All isolates were confirmed as GISA/hGISA by the National Reference Center for Staphylococci, Lyon, France by the reference method [10,18].

[b] GISA and hGISA phenotypes were not distinguished in this study because no strict delineation could be drawn between them, the heterogeneous phenotype being able to convert to the homogeneous phenotype and conversely depending on subculturing conditions [18].

[c] Unstable clinical status, pulmonary exacerbation except where specified.

[d] In this study, initial detection by antibiotype includes positive screening for decreased susceptibility to glycopeptides using routine agar diffusion assays (i.e., disk diffusion assay showing inhibition zone around the vancomycin or teicoplanin disk below the susceptibility breakpoint or inhibition diameter around the teicoplanin disk more than 3 mm smaller than that around the vancomycin disk or standard Etest MICs of vancomycin or teicoplanin >2 μg/ml) and/or observation of suggestive resistance to gentamicin and/or rifampin.

[e] KAN, kanamycin; TOB, tobramycin; ERY, erythromycin; LIN, lincomycin; TET, tetracycline; OFX, ofloxacin; FOF, fosfomycin; TMP, trimethoprim-sulfamethoxazole; BAL, bronchoalveolar lavage fluid sample; GSSA, glycopeptide-susceptible *S. aureus*; R, resistant; S, susceptible; NA, not applicable.

6. Case 5, a 12-year-old patient with GISA/MRSA colonization successfully treated by trimethoprim/sulfamethoxazole

In this patient, intermittent colonization by MRSA has been noted since the age of 7 years and colonization with *P. aeruginosa* was observed once at the age of 9 years. Despite colonization, lung function was stable (FEV_1 between 80 and 100% pred) and nutritional status was normal. At the age of 12 years (February 2010), a GISA/MRSA strain was isolated (Table 1). Despite a stable clinical status, co-trimoxazole (sulfamethoxazole 800 mg/trimethoprim 160 mg t.i.d.) was started for 15 days and no MRSA with decreased susceptibility to glycopeptides has been isolated to date in this patient.

7. Antimicrobial susceptibility testing and confirmation of decreased susceptibility to glycopeptides

Antimicrobial susceptibility testing was performed by disk diffusion assay performed and interpreted according to the 2010 guidelines of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/UserFiles/file/CASFM/casfm_2010.pdf). Standard vancomycin and teicoplanin MICs were determined by the Etest method (inoculum, 0.5 McFarland on Mueller-Hinton agar for 24 h; breakpoint for susceptibility, ≤ 2 μg/ml). Results are reported in Table 1. Decreased susceptibility to glycopeptides was confirmed for the eight isolates of this study by the National Reference Center for Staphylococci, Lyon, France using the population analysis profile–area under the curve (PAP-AUC) reference method [5,6].

8. Genotypic analysis and evidence for the absence of cross-contamination between patients

Using the DNA hybridization microarray Clondiag Staphy-Type® (Alere) and Multi Locus Sequence Typing (MLST) [7], we demonstrated: (i) the 2-year chronic colonization by a GISA strain belonging to the Iberian MRSA clone and displaying the following characteristics: ST 572, *SCCmec* I, *agr1*, *ccr* allotype A1B1, in patient 1, (ii) the successive isolation of two unrelated GISA/MSSA isolates in patient 2 (first isolate characteristics: ST5, *agr2*; second isolate characteristics: ST931, *agr1*), (iii) the absence of cross-contamination between the five patients, the strains displaying distinct genotypes (patient 3, MSSA, ST247, *agr1*; patient 4, strain belonging to the Lyon MRSA clone, ST8, *SCCmec* IV, *agr1*, *ccr* allotype A2B2; patient 5, strain belonging to the Cordobese MRSA clone, ST5, *SCCmec* I, *agr2*, *ccr* allotype A1B1).

9. Discussion

Since their first description in Japan in 1997, GISA and hGISA have been progressively reported worldwide [5,8-10]. These strains have been previously associated with prolonged antibiotic therapy, prolonged hospitalization, treatment failure, increased mortality and epidemic episodes with cross-contamination between patients [11]. In the routine practice of medical microbiology, their detection is problematic, particularly when strains displayed heterogeneous resistance and the isolates have to be confirmed as GISA/hGISA by a time-consuming reference method performed by a limited number of laboratories [12].

In CF patients, the first documented case of MRSA/GISA isolation was reported in an 18-year-old patient with pulmonary exacerbation who received successive courses of vancomycin therapy [2]. Then, Cafiso et al. reported three hGISA/MRSA strains in two CF patients (7 and 20 years old, respectively), i.e., in approximately 3% of CF patients harboring *S. aureus* [4], and Dumitrescu et al. reported the characterization of eight MRSA and one MSSA hGISA strains among 248 *S. aureus* isolates (3.6%) from 235 CF patients [3] but no clinical documentation is available for these cases. From these observations, it could be assumed that GISA/hGISA strains are emerging in CF patients. Here we found hGISA/GISA strains in 4.7% of the patients consulting the CF center of our institution and colonized/infected by *S. aureus* (5 out of 106 patients). Despite the few cases reported to date, some common characteristics between the cases reported here and the literature could be noted like persistence of GISA/hGISA strains in the airways of CF patients as well as successive isolation of strains with different phenotypic or genotypic characteristics [2,4]. To date, GISA/hGISA strains were demonstrated in CF patients with ages ranging from 7 to 36 years, colonized with *S. aureus* for at least 2 years and submitted to several courses of antimicrobial regimens not always including glycopeptides [2,4, this study]. Risk factors for GISA infection previously identified included vancomycin or teicoplanin treatment in the month and up to 6 months and MRSA isolation in the 3 months preceding the GISA isolation [13]. These factors are respectively noted for three and four patients in this study and documented for two and one cases reported in the literature, suggesting that common risk factors for selection of GISA strains might be observed for CF and non-CF patients. It is worth noting that CF patients are probably at high risk for GISA emergence because of modified pharmacokinetic and pharmacodynamic properties of antimicrobial drugs constituting favorable conditions for subinhibitory concentrations inducing the selection of strains with decreased susceptibility to glycopeptides [14,15]. However, some patients, like patient 2, did not receive glycopeptide treatment and/or were not colonized by MRSA, suggesting that other factors might be involved in the colonization/infection by GISA/hGISA strains in a non-outbreak setting but these factors still have to be elucidated.

Owing to the difficulty of GISA/hGISA detection, some previously reported microbiological characteristics might be helpful for their recognition beside failure to glycopeptide treatment. Most strains are MRSA, although decreased susceptibility to glycopeptides may also develop in MSSA strains, the latter representing less than 3% of GISA in France [6,16,17]. Resistance to gentamicin and rifampin has been previously reported in about half of the GISA/MRSA strains while rarely encountered in GSSA/MRSA (less than 5% of the strains in France) and may be considered as suggestive for associated decreased susceptibility to glycopeptides [13,18]. To our knowledge, only one GISA/MSSA isolate has been

previously reported in CF [3]. In this study, we report three additional GISA/MSSA beside five GISA/MRSA. We showed that resistance to gentamicin and/or rifampin could also be suggestive for decreased susceptibility to glycopeptides in MSSA, all strains being resistant or intermediate to rifampin and four strains, including the three clonal MRSA strains in patient 1 and the MSSA strain recovered in patient 3, being resistant to gentamicin. Finally, although this was not investigated by genotypic analyses in this study, some of the GISA isolates reported here may have arisen from MRSA strains as observed in previous studies [16,19]. Indeed, GISA/MRSA isolated in May 2007 in patient 1 was recovered in association with a second MRSA strain fully susceptible to glycopeptide after PAP-AUC analysis but showing an identical antimicrobial susceptibility pattern to the other antimicrobial agents, suggesting that the GISA/MRSA strain emerged from the GSSA/MRSA strain. Similarly, it is highly probable that the GISA/MSSA strain isolated in patient 3 derived from the MRSA strain colonizing the patient by excision of the chromosomal cassette containing the *mecA* gene because of an identical resistance pattern to agents other than β-lactams.

As in the first reported case, pulmonary exacerbation could be attributed at least in part to the hGISA/GISA strains in three out of the five patients in our study, thereby confirming the potential pathogenicity of these strains during CF [2]. However, we underlined that the clinical implication of hGISA/GISA strains during the course of CF remains particularly difficult to document because of the frequent concomitant isolation of several pathogens and the identification of hGISA/GISA strains in patients chronically colonized or infected by glycopeptide-susceptible *S. aureus* (GSSA). This was even more complicated here by the difficulty to date the initial GISA/hGISA colonization/infection due to unavailable isolates for retrospective analysis. Therapeutic implications of GISA strains are a matter of concern, particularly when MRSA strains with associated resistance to several other families of antimicrobial agents are involved [20]. No recommendations for treatment of GISA infections are currently published. Therapeutic success has been reported using high doses of vancomycin administered by continuous infusion and monitored by serum levels dosage in combination with fusidic acid followed by 3-month oral treatment with minocycline and fusidic acid [2]. In this study, linezolid, a second-line drug for management of respiratory exacerbation due to *S. aureus*, was successfully used in three patients whilst co-trimoxazole led to GISA eradication in one patient with stable clinical status [21]. However, these two antibiotics should be used with caution in CF patients, as they were previously involved in the emergence of linezolid-resistant and of persistent *S. aureus* (small-colony variants), respectively [22-24].

10. Conclusion

We report here the highest rate of GISA/hGISA described to date in the CF population, hGISA/GISA strains being found in 4.7% of the patients colonized/infected by *S. aureus*. However, these strains are probably underestimated because their detection remains difficult and additional studies including case–control studies are still needed to determine their incidence and clinical implication in CF patients as well as risk factors for acquisition. Despite growing knowledge on GISA/hGISA strains, such strains remain belatedly and/or retrospectively recognized in CF. Consequently, adapted antimicrobial treatment is usually delayed due to the time to result of the reference method used to confirm the resistance and colonized/infected patients may represent a reservoir for spread of GISA/hGISA strains in the meanwhile. Moreover, GISA detection in CF patients significantly narrows the therapeutic options and may worsen the clinical outcome. Due to the increasing cases reporting that these strains might be involved in exacerbation during CF, clinicians and microbiologists should remain aware of *S. aureus* isolates with particular antimicrobial susceptibility profiles and/or patients with poor clinical course or treatment failure and should perform GISA/hGISA detection more systematically.

Acknowledgments

Thanks are extended to Philippe Bret, Pfizer and to the society KRAUS BIOMEDICAL who helped to write the manuscript in English, and to Fabien Aujoulat and Annie Martra for their excellent technical assistance. This study has been supported by the association ADEREMPHA, Sauzet, France.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jcf.2011.05.001.

References

[1] Filleron A, Chiron R, Jean-Pierre H, Reverdy ME, Aleyrangues L, Counil F, Jumas-Bilak E, Marchandin H. *Staphylococcus aureus* with decreased susceptibility to glycopeptides in cystic fibrosis patients. 32nd European Cystic Fibrosis Society Conference, Brest, France, 10–13 June 2009.

[2] Denis O, Nonhoff C, Byl B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of vancomycin-intermediate *Staphylococcus aureus* in a Belgian hospital: microbiological and clinical features. J Antimicrob Chemother 2002;50:383–91.

[3] Dumitrescu O, Reverdy ME, Bes M, et al. Étude phénotypique et génotypique de 9 souches de *Staphylococcus aureus* de sensibilité diminuée aux glycopeptides, isolées chez des enfants atteints de mucoviscidose. 29th Réunion Interdisciplinaire de Chimiothérapie Anti-infectieuse, Paris, France, 3–4 December 2009.

[4] Cafiso V, Bertuccio T, Spina D, et al. Methicillin resistance and vancomycin heteroresistance in *Staphylococcus aureus* in cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 2010;29:1277–85.

[5] Hiramatsu K, Aritaka N, Hanaki H, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 1997;350:1670–3.

[6] Bobin-Dubreux S, Reverdy ME, Nervi C, et al. Clinical isolate of vancomycin-heterointermediate *Staphylococcus aureus* susceptible to methicillin and in vitro selection of a vancomycin-resistant derivative. Antimicrob Agents Chemother 2001;45:349–52.

[7] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008–15.

[8] Al-Obeid S, Haddad Q, Cherkaoui A, Schrenzel J, François P. First detection of an invasive *Staphylococcus aureus* strain (D958) with reduced

susceptibility to glycopeptides in Saudi Arabia. J Clin Microbiol 2010;48: 2199–220.

[9] Centers for Disease Control and Prevention (CDC. Staphylococcus aureus with reduced susceptibility to vancomycin—United States, 1997. MMWR Morb Mortal Wkly Rep 1997;46:765–6.

[10] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 1997;40:135–6.

[11] Rong SL, Leonard SN. Heterogeneous vancomycin resistance in *Staphylococcus aureus*: a review of epidemiology, diagnosis, and clinical significance. Ann Pharmacother 2010;44:844–50.

[12] Conly JM, Johnston BL. VISA, hetero-VISA and VRSA: the end of the vancomycin era? Can J Infect Dis 2002;13:282–4.

[13] Fridkin SK, Hageman J, McDougal LK, et al. Vancomycin-Intermediate *Staphylococcus aureus* Epidemiology Study Group. 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.

[14] Liu C, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 2003;47:3040–5.

[15] Macfarlane M, Leavy A, McCaughan J, Fair R, Reid AJ. Successful decolonization of methicillin-resistant *Staphylococcus aureus* in paediatric patients with cystic fibrosis (CF) using a three-step protocol. J Hosp Infect 2007;65:231–6.

[16] Pillai SK, Wennersten C, Venkataraman L, Eliopoulos GM, Moellering RC, Karchmer AW. Development of reduced vancomycin susceptibility in methicillin-susceptible *Staphylococcus aureus*. Clin Infect Dis 2009;49: 1169–74.

[17] Garnier F, Chainier D, Walsh T, et al. A 1 year surveillance study of glycopeptide-intermediate *Staphylococcus aureus* strains in a French hospital. J Antimicrob Chemother 2006;57:146–9.

[18] Howe RA, Monk A, Wootton M, Walsh TR, Enright MC. Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. Emerg Infect Dis 2004;10:855–7.

[19] Noto MJ, Fox PM, Archer GL. Spontaneous deletion of the methicillin resistance determinant, *mecA*, partially compensates for the fitness cost associated with high-level vancomycin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 2008;52:1221–9.

[20] Liñares J. The VISA/GISA problem: therapeutic implications. Clin Microbiol Infect 2001;7(Suppl 4):8–15.

[21] Ferrin M, Zuckerman JB, Meagher A, Blumberg EA. Successful treatment of methicillin-resistant *Staphylococcus aureus* pulmonary infection with linezolid in a patient with cystic fibrosis. Pediatr Pulmonol 2002;33:221–3.

[22] Gales AC, Sader HS, Andrade SS, Lutz L, Machado A, Barth AL. Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. Int J Antimicrob Agents 2006;27: 300–2.

[23] Zander J, Besier S, Saum SH, et al. Influence of dTMP on the phenotypic appearance and intracellular persistence of *Staphylococcus aureus*. Infect Immun 2008;76:1333–9.

[24] Besier S, Smaczny C, von Mallinckrodt C, et al. Prevalence and clinical significance of *Staphylococcus aureus* small-colony variants in cystic fibrosis lung disease. J Clin Microbiol 2007;45:168–72.