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Abstract

Staphylococcus aureus with resistance to glycopeptide antibiotics has been considered to be a rare cause of clinically relevant infections. A review of the current literature shows that this is indeed the case for infections caused by *S. aureus* with high-level resistance to vancomycin (VRSA), as only isolated cases have been reported. VRSA develops following the insertion of the vanA gene, which is transferred from enterococci with vancomycin resistance. On the other hand, infections caused by *S. aureus* with intermediate resistance to glycopeptides (VISA), or heterogeneously expressed intermediate level glycopeptide resistance (hVISA), are more common. These infections are associated with clinical failure of glycopeptide therapy. While the biochemical and phenotypic features including a thickened cell wall of hVISA and VISA are well known, the genetic basis of these phenotypes remains unknown. Certain genetic regulatory elements such as *agr* II are associated with reduced susceptibility of *S. aureus* to glycopeptides. Available data suggest that certain infections might be successfully treated using higher doses of vancomycin. However, as treatment failure is particularly common in infections with a high bacterial load, it may be necessary to resort to other antibiotics such as linezolid, often combined with surgical intervention, in order to successfully treat these infections. Open questions regarding diagnosis, pathogenesis, epidemiology, and treatment of glycopeptide resistance in *S. aureus* are addressed in this review. Clinicians should be aware of these aspects, since *S. aureus* remains one of the most important bacteria in modern medicine.

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Introduction

Infections caused by antibiotic-resistant bacteria are a growing problem in many geographic areas [1]. Hospitalized patients as well as outpatients are affected by this problem [2]. In adult outpatients, the prevalence of resistance of *Escherichia coli* to aminopenicillins or quinolones, of *Streptococcus pneumoniae* to penicillin or macrolides, and of *Streptococcus pyogenes* to macrolides is relatively high in various geographic areas [3]. Similarly, antibiotic resistance of *S. pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, and *Moraxella catarrhalis* has become a clinically relevant concern regarding the treatment of infections in children in most countries [4], whereas *Staphylococcus aureus* has typically been susceptible to antibiotics that are used to treat infections in the outpatient setting. Following the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA), this susceptibility can no longer be assumed in several areas of the world [5]. Infections caused by antibiotic-resistant bacteria may have a protracted or even lethal course [6, 7].

S. aureus is probably one of the most common pathogens in outpatient as well as hospital medicine. Infections caused by *S. aureus* may be relatively mild and easy to treat, or may take a dramatic, and at times life-threatening course, as is illustrated by numerous case reports and studies [8,9]. In addition to *S. aureus*, other staphylococcal species such as *Staphylococcus lugdunensis* have a high pathogenic potential due to the expression of binding factors and may cause severe infections such as rapidly destructive endocarditis [10, 11].

Antibiotic resistance in *S. aureus* may have negative consequences in several areas of medicine. Probably the most visible consequence of the increased resistance is seen in the clinical management of individual patients with infections caused by resistant *S. aureus*. Methicillin resistance of *S. aureus* will force clinicians to resort to antibiotics such as vancomycin that are less bactericidal, or more slowly bactericidal than betalactam antibiotics such as nafcillin [12]. The *in vitro* observation of a reduced bactericidal effect of vancomycin is clinically relevant, as has been shown in a prospective study of the treatment of bacteremia caused by methicillin-susceptible *S. aureus* (MSSA)

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Table 1
Breakpoints (µg/ml) for MIC testing of *S. aureus*.

Organization	Vancomycin Susceptible	Vancomycin Intermediate	Vancomycin Resistant	Teicoplanin Susceptible	Teicoplanin Intermediate	Teicoplanin Resistant
NCCLS	≤ 4	8–16	≥ 32	≤ 8	16	≥ 32
SFM	≤ 4	8–16	≥ 32	≤ 4	8–16	≥ 32
BSAC, SRGA	≤ 4	–	≥ 8	≤ 4	–	≥ 8

NCCLS: National Committee for Clinical Laboratory Standards; SFM: Société Française de Microbiologie; BSAC: British Society for Antimicrobial Chemotherapy; SRGA: Swedish Reference Group for Antibiotics

[13]. In this study, the relapse rate following treatment with vancomycin was significantly higher than the corresponding rate after treatment with nafcillin.

Several years ago, the problem of antibiotic resistance of *S. aureus* was accentuated by the appearance of clinical infections caused by strains that exhibited resistance to glycopeptides [14–16]. A series of investigations has shown that glycopeptide resistance of *S. aureus* is not a "yes-no" phenomenon, but includes intermediate levels of resistance as well as strains, which upon more detailed investigation are heteroresistant.

This review will summarize the current epidemiology of glycopeptide resistance in *S. aureus*, its trends, and will focus on clinical issues such as diagnosis and treatment of infections caused by *S. aureus* with heteroresistance, intermediate or complete resistance to glycopeptides. Furthermore, published experiences on modes of transmission of these bacteria in the hospital and in the outpatient setting, as well as preventive measures will be summarized.

Categories of Glycopeptide Resistance

Reduced susceptibility to vancomycin has been reported repeatedly over the course of the last few years. The NCCLS defines *S. aureus* isolates with vancomycin MICs between 8 and 16 µg/ml as intermediately sensitive, and isolates with a MIC of ≥ 32 µg/ml as resistant, whereas in Japan the breakpoint for resistance is ≥ 8 µg/ml [17]. Similarly, British and Swedish definitions do not include a category of intermediate susceptibility to vancomycin or teicoplanin (Table 1) [18]. Strains of *S. aureus* with a heterogeneous population regarding the susceptibility to vancomycin are termed heterogeneous vancomycin (or glycopeptide) intermediate *S. aureus* (hVISA or hGISA) [18].

Hiramatsu et al. [19], who were first to report a heteroresistant clone, labeled Mu3, defined a *S. aureus* strain as heteroresistant, if subclones with a vancomycin MIC of ≥ 8 µg/ml were produced upon selection with vancomycin. Such strains had to remain stable at least 9 days in a drug-free medium. More recently, *Walsh* and *Howe* modified the definition of heteroresistance by requiring that population heterogeneity should be demonstrated in a full population analysis profile [18]. According to these authors, a *S. aureus* isolate is defined as heteroresistant to vancomycin, if the ratio of the areas under the population analysis curve of the isolate in question and of the Mu3 control strain is ≥ 0.90.

This laborious diagnostic approach is not practical for routine microbiology laboratories. On the other hand, the sensitivity of the broth microdilution method is insufficient to reliably detect hGISA [20, 21]. The Etest method with a large inoculum (no. 2 McFarland standard) and an extended incubation time (48 h) was found to be a sensitive screening method for the detection of glycopeptide resistance [22]. However, low rates of false-positive results – 1.6% [20] and 2.1% [22] – have been reported. The sensitivity and specificity of Etest screening on brain heart infusion agar using 2.0 McFarland inocula and a breakpoint of ≥ 8 µg of vancomycin per ml compare favorably to other methods as shown in table 2 [22]. Current CDC recommendations are not based on the use of a high inoculum. The proposed algorithm for testing of *S. aureus* regarding vancomycin susceptibility starts with either a nonautomated MIC method (reference broth microdilution, agar dilution, or Etest using a 0.5 McFarland standard inoculum on Mueller-Hinton agar), or with disk diffusion combined with a vancomycin screen plate (BHIA with 6 µg/ml of vancomycin) [23]. A comparison of methods used in published studies to diagnose heteroresistance of *S. aureus* to glycopeptides shows that definitions and conditions for laboratory detection of hGISA are not standardized. This may result in an underestimation of the true prevalence of such strains among

Table 2
Sensitivity and specificity of various methods to detect heteroresistance or intermediate resistance to vancomycin in MRSA [22].

Method	Sensitivity (%)	Specificity (%)
Agar dilution	20	100
Broth dilution	11	100
Mueller-Hinton screening	20	99
BHI screening	22	97
Simplified population screening	71	88
Etest, 0.5 McFarland	82	93
Etest 2.0 McFarland	96	97

Table 3
Pathogenesis of glycopeptide resistance in *S. aureus*.

Type of resistance	Genetic event	Biochemical and phenotypic effects	Reference
VRSA	Acquisition of vanA gene (transposon Tn1546) from vancomycin-resistant *Enterococcus faecalis*	Replacement of the carboxy terminal D-alanyl-D-alanine of the peptidoglycan cell wall precursor by D-alanyl-D-lactate; resulting in a 1,000-fold lower affinity to vancomycin	*Weigel* [29]
hVISA, VISA	Unknown	Thickening of cell wall secondary to: · Increased production of peptidoglycan · Reduced turnover of peptidoglycan Resulting in: · Affinity trapping of vancomycin · Clogging phenomenon by trapped vancomycin · Raised proportion of D-alanyl-D-alanine residues in peptidoglycan layers further increasing trapping of vancomycin	*Hiramatsu* [32]

clinical isolates of *S. aureus* [24], and renders comparison of epidemiological data difficult.

While the Etest with a higher inoculum appears to improve the sensitivity of the diagnostic approach to heteroresistance in *S. aureus*, care must be taken to avoid the artificial elevation of the vancomycin MIC by serial passage of the bacteria on media containing vancomycin. This effect was already described in the mid-1950s, as pointed out by *Moellering* [25]. Despite recent progress in laboratory approaches, detection of heteroresistant *S. aureus* in the routine clinical microbiology laboratory remains a difficult challenge, as definitive proof still requires population analysis profiling [19].

Pathogenesis

The pathogenesis of resistance of *S. aureus* to glycopeptides is not fully understood. Except for rare cases, reduced susceptibility to glycopeptides is not found in MSSA. So far, a single case of MSSA with heteroresistance to glycopeptides has been reported [26, 27]. The genetic events resulting in phenotypic expression of resistance appear to be different in strains with high-level vancomycin resistance compared with strains exhibiting intermediate levels of resistance (Table 3). *S. aureus* with reduced susceptibility to vancomycin has a thick cell wall in comparison to susceptible *S. aureus* [28], whereas vancomycin-resistant *S. aureus* is resistant as a result of the acquisition of the vanA gene from vancomycin-resistant enterococcus [29]. This gene is then integrated into a *S. aureus* conjugative plasmid. Vancomycin resistance is only expressed when the bacterial cell is exposed to vancomycin [30]. In addition, vancomycin- and methicillin-resistance may be co-expressed in VRSA strains, suggesting that resistance may also occur in the absence of selection pressure caused by vancomycin use [31].

In *S. aureus* with intermediate resistance to glycopeptides, cell-wall thickness plays a major role in mediating resistance. As reviewed by *Hiramatsu* [32], the first clinical *S. aureus* strain with reduced susceptibility (Mu50) has 30–40 layers of peptidoglycan compared to approximately 20 layers in fully susceptible strains. The increased number of layers in VISA contains many D-alanyl-D-alanine targets to which glycopeptide molecules can bind (affinity trapping), thus resulting in a reduced access of glycopeptides to their site of action, namely the D-alanyl-D-alanine residues of murein monomers, which are located in the cytoplasmic membranes, and are ready to be used in peptidoglycan synthesis. If the glycopeptides bind to these monomers, peptidoglycan synthesis, and as a consequence cell multiplication, is completely inhibited [32]. Penetration of glycopeptides to their site of action in the cytoplasmic membrane is further inhibited by the destruction of the mesh structure of the outer layers of peptidoglycan by the trapped glycopeptide molecules themselves. This event was described as "clogging phenomenon" by *Cui* et al. [33].

The genetic basis for the regulation of cell-wall thickness in *S. aureus* is currently not known. The presence of vancomycin or beta-lactams in the bacterial environment appears to influence this phenotypic expression. In a recent review, *Hiramatsu* [32] favored the hypothesis that heteroresistant VISA is a precursor to VISA and that these two phenotypes are expressed dependent on the selection pressure of vancomycin. This concept is illustrated in figure 1, which is adapted from *Hiramatsu's* review. Further

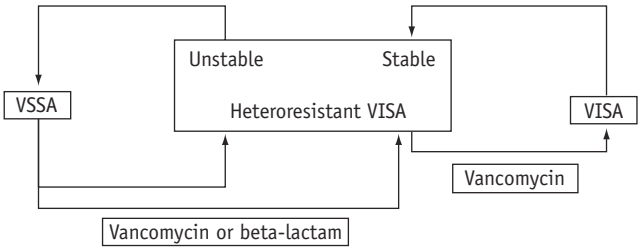

Figure 1. Phenotypic stages of MRSA dependent on presence or absence of selection pressure by vancomycin or beta-lactam antibiotics (adapted from [32]).

research will be needed in order to assess the validity of this hypothesis.

Regulation of resistance to vancomycin is probably under the control of several genes. Among these, downregulation of tcaA, which encodes a transmembrane protein, was associated with increased glycopeptide resistance in *S. aureus* [34]. Another regulatory gene, the accessory gene regulator *(agr)* of *S. aureus* also appears to play a role in the expression of glycopeptide resistance. This gene occurs in four groups. *Sakoulas* et al. [35] have recently shown that *agr* group II was more prevalent in blood culture isolates of MRSA than MSSA. The authors performed population analysis of genetically engineered *agr*-null *S. aureus* strains and were able to show that glycopeptide heteroresistance is associated with loss of *agr* function. In an earlier study, the authors showed that GISA and hetero-GISA strains from the United States and Japan belonged to *agr* group II and that many GISA isolates were defective in *agr* function [36]. Based on these and other studies, *Walsh* and *Howe* [18] hypothesize that mutations in *agr* and/or sar, another regulatory locus of *S. aureus*, or altered expression of these regulators, may lead to a VISA or hVISA phenotype.

Epidemiology of Glycopeptide Resistance

Infections caused by *S. aureus* with high-level resistance to vancomycin are rare. Only three cases have been reported so far [37–41]. All three patients had underlying diseases, had received antibiotics, and were reported from the USA. Coinfection with MRSA and VRE was present.

Intermediate resistance of *S. aureus* to vancomycin is clearly more common than high-level resistance. At least 20 cases of VISA infections have been reported from various continents [18]. Pretreatment with vancomycin was a common feature in many of these patients [42, 43].

The prevalence of *S. aureus* with heteroresistance to glycopeptides appears to be higher than the corresponding prevalence of VISA. Several studies have been conducted in Europe (France [27, 44–49], Germany [50, 51], Italy [52], Netherlands [20], Spain [53], United Kingdom [54, 55]), Asia – Hong Kong [56], Japan [19, 57, 58], Korea [59, 60]), South America – Brazil [61]), and North America – USA [62–64]). Reported prevalence rates vary greatly between, as well as within continents (Table 4). These results should be interpreted with caution, as methods to screen for and to diagnose hVISA varied widely from study to study.

Table 4
Geographic variation of the prevalence of heteroresistance to vancomycin in *S. aureus*.

Geographic area	Prevalence rate (range, %)
Europe	0–27[a]
Asia	0–26[b]
Brazil	3
USA	0–3.1

[a]Most studies from Europe reported rates between 0 and 5%. A high rate of 27% was found in liver transplant recipients infected or colonized with MRSA [49]. [b]Conflicting results were reported from Japan. While *Hiramatsu* reported a prevalence rate of 26% in four university hospitals, where the study was conducted in 1996 [19], *Ike* failed to detect any heterogeneously or intermediately resistant strains among 6,625 clinical MRSA isolates collected in 278 hospitals throughout Japan during 1997 [58].

In many other areas the prevalence of vancomycin resistance appears to be low. A recent prevalence survey of more than 1,000 MRSA isolates in Belgium found only one homogeneous vancomycin-resistant *S. aureus* (VISA) and five heterogeneous VISA (hVISA) based on population analysis profiling [65]. In a Brazilian study of 140 MRSA isolates, five *S. aureus* with reduced susceptibility to vancomycin (MIC 8 µg/ml) were found [66]. None were positive for vanA and all had thickened cell walls.

The Netherlands have a very low prevalence of MRSA. As heteroresistance to vancomycin appears to be associated with vancomycin use to treat MRSA infections, heteroresistance would not be expected to be prevalent in countries with such a low prevalence of MRSA. However, *van Griethuysen* et al. [20] reported a rate of 6% of heteroresistance to vancomycin in MRSA strains. Epidemiological information on the origin of the affected patients revealed that none of the isolates originated the Netherlands. The patients were in Turkey, Greece, Italy, France, Germany, and the Ivory Coast.

Risk Factors for Glycopeptide Resistance

According to the hypothesis of *Hiramatsu* [32] (Figure 1), use of glycopeptides or beta-lactam antibiotics is a major driving force of glycopeptide resistance in *S. aureus*. Indeed, the analysis of published cases of infections caused by VISA or hVISA reveals that the majority of patients has a history of pretreatment with glycopeptides and/or beta-lactams. However, in patients with hVISA, vancomycin was often used to treat the infectious episode during which hVISA was recognized. This observation also supports *Hiramatsu's* concept of an induction of an unstable or stable phenotype of hVISA by ongoing glycopeptide therapy.

As glycopeptides are the first choice of clinicians for the treatment of infections caused by methicillin-resistant staphylococci, the use of these agents (vancomycin, teicoplanin) is fairly widespread. In a 3-year survey of eight German university hospitals, performed between 1998 and 2000, *Kern* et al. [67] registered an average glycopeptide use ranging between 1.3 and 8.8 DDD/100 patient days on medical wards, whereas the corresponding use on surgical wards ranged between 0.7 and 1.8 DDD/100 patient days. Compared to an earlier survey [68], which was performed in four of these hospitals, glycopeptide use on surgical wards was lower in some hospitals, probably as a result of an active antibiotic management program. Glycopeptide use was markedly higher in intensive care units and hematology-oncology units, ranging up to 15.7 DDD/100

patient days. There was a striking variation in glycopeptide use between hospitals, which did not correlate with differences in the prevalence of endemic MRSA. Differences in glycopeptide use between medical, surgical, and intensive care units were also observed in an Italian point prevalence survey of antibiotic consumption in 15 hospitals in 2001 [69], and in another German survey of antibiotic use in ICUs, with lower use rates in medical compared to surgical ICUs [70].

Anecdotal reports of the development of heteroresistance to vancomycin in MRSA during prolonged vancomycin therapy suggest that the prevalence of heteroresistant VISA may increase in institutions with a high use of vancomycin [24]. Heteroresistance to vancomycin may also be induced by the treatment of MRSA infections with a cephalosporin in combination with vancomycin, as seems to be a common practice in Japan [71].

Antibiotic use is not only a concern in adult patients. A recent study by *Potocki* et al. [72] showed that 36% of hospitalized children received antibiotics during their stay. Overall, 15% of antibiotic use was judged inappropriate.

While antibiotic resistance in *S. aureus* is a major problem in many European countries, resistance in other bacterial species should also be considered, as exchange of resistance plasmids has been observed. Linezolid is a valid alternative to vancomycin, and might be one of the few remaining antibiotics with activity against vancomycin-resistant *S. aureus*. However, transfer of linezolid resistance from enterococci to *S. aureus* may result in linezolid resistance in *S. aureus* as well. Clinically relevant linezolid resistance has been reported in isolated cases [73]. Surveillance of antibiotic resistance should therefore also include surveillance of linezolid resistance in gram-positive cocci in general.

In addition to glycopeptide or beta-lactam use, other clinically relevant risk factors for the development of glycopeptide resistance in *S. aureus* are frequently present. These are summarized in table 5. A high bacterial load infection in a patient with a serious underlying disease, who is receiving glycopeptide treatment for a MRSA infection at dosages that result in low concentrations, is probably the prototype scenario for the emergence of the hVISA or VISA phenotype during therapy.

Clinical Impact of Glycopeptide Resistance

Resistance to antibiotics does not necessarily result in a worse outcome, as has been demonstrated in several studies. There was no correlation between inappropriate antibiotic treatment as a result of resistance and mortality in patients with *Citrobacter freundii* bacteremia [74]. This unexpected observation can be explained by the presence of underlying diseases and other clinical parameters, which also have a major impact on outcome.

Therefore, adjustment for confounding variables needs to be made when comparing outcomes of infections caused by resistant or susceptible bacteria. The treatment of MRSA infections is already difficult, since only a limited number of antibiotics are active against MRSA. A meta-analysis of studies examined the difference in mortality between MRSA and methicillin-susceptible *S. aureus* bacteremia [8]. The pooled risk of death from MRSA bacteremia for all 31 studies was almost 2-fold higher than the risk of dying from MSSA bacteremia.

MRSA infections are particularly difficult to treat, if they are located at anatomical sites, where antibiotic penetration is reduced, such as the central nervous system. Bacterial meningitis caused by MRSA was associated with a mortality rate of 56%, compared to a mortality rate of 13% in a patient group with meningitis caused by methicillin-susceptible *S. aureus* [75]. All patients with MRSA infections were treated with vancomycin, which penetrates poorly into the cerebrospinal fluid. In several patients with MRSA vertebral osteomyelitis treatment with vancomycin failed to eradicate the infection [76]. All isolates had MICs to vancomycin of 1 μg/ml or less by routine susceptibility testing; however heteroresistance was not excluded. In addition to pharmacokinetic reasons, the presence of heteroresistance to *S. aureus* should be considered in these patients, since all had received several weeks of vancomycin treatment prior to the culture, which documented treatment failure.

Clinical failures have been reported in patients with infections caused by VISA or hVISA [43, 77]. Accessory gene regulator group II polymorphism in MRSA has been found to be an independent predictor of vancomycin treatment failure [78]. How this polymorphism leads to vancomycin failure is currently not known. However, treatment failure may not always be caused by the resistance problem, but may be the result of clinical circumstances, such as an undrained abscess. The true clinical relevance of heteroresistance or intermediate resistance to glycopeptides in *S. aureus* still remains a matter of debate. Outcomes of

Table 5
Risk factors for infections caused by hVISA or VISA.

Risk factor	Reference
Glycopeptide use	
· Widespread use: selection pressure	*Hiramatsu* [32]
· Low tissue concentration	*Charles* [79]
· Vancomycin treatment failure	*Charles* [79]
Underlying disease	*Howden* [82]
· Diabetes mellitus	
· Immunosuppression	
· Malignancy	
· End-stage renal failure	
· Surgery within 8 weeks before hVISA detection	
High bacterial load infection	*Charles* [79],
· Endocarditis	*Ariza* [53]
· Abscess	
· Orthopedic device infection	

published cases vary greatly. Many reports do not provide sufficient details to assess the role of the infection itself, or of the antibiotic treatment in the clinical course of individual patients.

Infections caused by heteroresistant VISA are associated with a protracted clinical course, as has been shown in a study of hVISA bacteremia, which persisted for 7 days more in a larger proportion of patients than did MRSA bacteremia [79]. Therefore, reduced susceptibility to vancomycin should be suspected in patients with MRSA infection that does not improve despite adequate vancomycin levels [80]. In a study of 22 patients with persistent or recurrent MRSA bacteremia, heteroresistance to vancomycin was found in three (13.6%) isolates, suggesting that heteroresistance may be a relevant cause of treatment failure [81].

Treatment Options for hVISA or VISA

At least some of the reported cases appear to demonstrate a favorable clinical response to vancomycin treatment. However, a recent study by *Howden* et al. [82] showed that glycopeptide therapy failed for 76% of the patients. Only ten out of 21 patients were cured using other antibacterial agents, and 60% of the patients required surgery. It therefore appears prudent to aim for higher serum trough vancomycin levels (15–20 µg/ml) during treatment of hVISA infections, even though the MIC of vancomycin for most strains that were identified as VISA or hVISA is well below the serum levels, which are normally achieved during treatment with vancomycin [83]. It must also be kept in mind that achievable vancomycin concentrations in certain anatomical locations such as the lung or in abscesses are markedly lower than the corresponding serum concentrations [84, 85].

As recurrent episodes of hVISA bacteremia have been reported despite adequate serum vancomycin levels [56, 86], other treatment options are needed to treat refractory infections. Among these, quinupristin/dalfopristin has been studied clinically in various settings. A recent report documented that this antibiotic can be successfully used to treat severe MRSA infections in patients with end-stage renal insufficiency [87]. However, its penetration into the CSF is very limited [88]. There are no reports describing the efficacy of quinupristin/dalfopristin for the treatment of hVISA or VISA infections.

Linezolid has been used successfully to treat meningitis [89-91], as well as osteomyelitis caused by MRSA [92]. It has also been used successfully to treat hVISA infections after vancomycin failed [82]. Median duration of treatment was 41 days.

Antibiotic combinations may be another option to approach clinical failures due to hVISA or VISA. In a non-controlled, observational study of five patients with methicillin-resistant *S. aureus* or coagulase negative staphylococcal infections who had previously failed treatment with vancomycin, the combination of quinupristin/dalfopristin with a glycopeptide resulted in clinical cure in four patients [93]. *In vitro* testing of most bacterial isolates revealed synergy between quinupristin/dalfopristin and teicoplanin.

In their study of treatment outcomes in hVISA infections, *Howden* et al. [82] used the combination of rifampicin and fusidic acid following clinical control of the infection by linezolid. One patient with bacteremia and septic arthritis caused by a hVISA isolate with resistance to rifampin was treated with fusidic acid and chloramphenicol after initial treatment with linezolid. *Wong* et al. [86] reported a favorable clinical response in a patient with bacteremia after adding fusidic acid to the regimen. Based on *in vitro* checkerboard testing, these authors also demonstrated synergy between ampicillin and vancomycin, despite the fact that over 90% of staphylococci studied were producers of penicillinase. *Heym* et al. [94] treated five patients with infections caused by S. *aureus* with intermediate resistance to teicoplanin, using a combination of vancomycin, fusidic acid, and chloramphenicol (three patients, all cured), or with vancomycin combined with fusidic acid, or vancomycin alone (one patient each).

An *in vitro* study of vancomycin combined with cephalosporins in a clinical isolate of *S. aureus* that exhibited heteroresistance to vancomycin (MIC < 8 µg/ml), yielded nonconclusive results regarding the inhibitory effect of such a combination [95]. Although no randomized, clinical studies of the treatment of infections caused by hVISA or VISA have been conducted, currently available data suggest that linezolid may be a good alternative to vancomycin or teicoplanin for the treatment of these infections. As isolated cases of linezolid-resistant MRSA or VRE have been reported, this new therapeutic agent should be used judiciously to preserve its clinical activity [96–98].

Treatment options are even more limited for infections caused by *S. aureus* with resistance to vancomycin. The vancomycin MIC of the Michigan isolate [37] was 1,024 µg/ml and the strain was also resistant to aminoglycosides, beta-lactams, fluoroquinolones, macrolides, rifampin, and tetracycline [29]. This scenario is threatening and must be addressed in a comprehensive fashion in order to limit its clinical and public health consequences [99, 100].

Infection Control Issues

Modes of Transmission of VISA and hVISA

S. aureus with heteroresistance to vancomycin may cause outbreaks, as has been shown by *Cassone* et al. [101], who described the transmission of a clone among patients and healthcare workers in a cardiac surgery ICU. This clone was genetically related to a previously known MRSA clone. Other authors also provide some circumstantial evidence for the nosocomial transmission of such strains (Table 6). It seems probable that the same modes of transmission apply to these strains, which are well described for MRSA. However, no studies have examined the biological behavior of VISA and hVISA regarding transient hand colonization of healthcare workers (HCW), nasal and skin colonization

of patients and HCW, survival of these strains on environmental surfaces, degree of environmental contamination related to patients with colonization or infection by these phenotypes of *S. aureus*.

Infection Control Measures

Given the pathogenetic model of phenotypic transition from VSSA to hVISA and eventually VISA under antibiotic selection pressure, it follows that reduction of glycopeptide or beta-lactam use is a central and crucial measure to reduce the risk for an increase in the prevalence of hVISA and VISA [32]. Perioperative antibiotic prophylaxis is one of the major indications for antibiotic use. Guidelines for the use of antibiotics in this setting exist and are used by many hospitals. Most guidelines recommend first or second-generation cephalosporins for the majority of indications of perioperative antibiotic prophylaxis. This recommendation may need to be revised in settings with a very high rate of MRSA. Despite existing guidelines, which recommend applying prophylaxis as a single dose, misuse of antibiotics in this setting is fairly common. A survey of German hospitals found that prophylaxis was limited to a single dose in less than half of the procedures [102]. As both the prophylactic and therapeutic use of antibiotics will exert a selective pressure on the bacterial flora of exposed patients, it will be important to improve the prophylactic use of antibiotics in order to reduce the risk of resistance development in the hospital setting [103]. A recent report of a patient with community-acquired pneumonia caused by a levofloxacin-resistant *S. pneumoniae* illustrates the clinical relevance of any use of antibiotics, as this may result in inadvertent development of resistance in bacteria, which were not the target of the antibiotic prescription [104]. Similarly, a Dutch study found a parallel increase in the prevalence of quinolone-resistant *E. coli* and in the use of fluoroquinolones among Dutch outpatients [105].

In addition to efforts to curtail misuse of antibiotics in the hospital, it will be important to apply a comprehensive strategy to reduce the total number of MRSA. According to *Hiramatsu* [32], this is the most effective measure for preventing emergence of VISA and hVISA. *Cosgrove* et al. [17] recently reviewed the CDC recommendations for infection control for patients infected or colonized with *S. aureus* with decreased susceptibility to vancomycin. These recommendations (isolation in a private room, gowns, gloves to enter room, mask and eye protection if aerosolization is possible, hand disinfection, avoidance of sharing of equipment between patients, minimization of the number of staff caring for patient, staff education) do not differ from measures recommended to prevent transmission of MRSA. In addition to these measures, the CDC recommends to perform weekly screening of the nares of

Table 6
Studies reporting nosocomial transmission of hVISA or VISA.

Country [Reference]	Setting	No. of patients involved	Glycopeptide pretreatment	Remarks
France [126]	Acute care and long-term care facility (LTCF)	15	None	11 patients from LTCF (10 nasal colonization, 1 UTI), 3 from ICU (pulmonary infection); 2 genotypes by PFGE.
Italy [52]	Rehabilitation unit	2	2	Epidemiological evidence suggestive of transmission. However, genetically related MRSA clone is widely disseminated in several units.
France [127]	Acute care	39	Unknown	High degree of genetic relatedness, same rare lysotype, multiresistant phenotype; increase in frequency over 2 month period as arguments for transmission. No further data to assess true rate of transmission.
France [94]	Acute care	6	1/6	Epidemiological evidence linking patients, results of PFGE and multilocus sequence typing suggesting transmission of MRSA with reduced susceptibility to teicoplanin.
France [46]	Acute care	6	Unknown	Single clone; transmission postulated without giving detailed information.
France [49]	Liver transplant recipients	13	2/13	11 strains belonging to same genetic cluster. Preoperative screening negative. Authors conclude from this and the absence of glycopeptide exposure that transmission was responsible for these cases.

persons with extensive patient contact [17]. In addition, isolates with vancomycin MICs of 4 mg/l or higher should be reported to local and state health authorities [17].

From a theoretical point of view, it could be hypothesized that the thick cell wall of VISA and hVISA strains would render the bacteria relatively resistant to chemical or physical means of inactivation such as certain disinfectants. Indeed, a recent report described a high rate (> 84%) of resistance to triclosan in French GISA isolates [106]. Increased MICs of chlorhexidine, benzalkonium chloride, and hexanidine di-isoethionate were also found.

Even in high-level vancomycin-resistant *S. aureus*, the host plasmid is a multiresistance plasmid, which even confers resistance to quaternary ammonium compounds [29]. It is currently unclear whether these *in vitro* observations are relevant and will have an impact on recommendations for hand, instrument, or surface disinfection. This will have to be determined by additional investigations.

The Cost of Antibiotic Resistance

Measurement of the impact of antibiotic resistance on healthcare costs in various countries is difficult due to imprecision of data about several parameters, such as resistance rates, antibiotic consumption data, outcome measures, and data on the incidence of nosocomial infections [107]. However, as the resistance problem is growing, it may be necessary to include resistance data into reports of national surveillance systems of nosocomial infections, in order to assess and compare performances and risks of hospitals regarding these infections [108]. Such a system has been designed and implemented in more than 30 German intensive care units [70, 109].

The results of some local studies provide interesting information regarding the negative consequences of antibiotic resistance on cost. A study from Duke University Medical Center reported median total costs for nosocomial bacteremia caused by MRSA of $27,083 versus $9,661 for bacteremia caused by methicillin-sensitive *S. aureus* [110]. A systematic audit of studies describing economic aspects of nosocomial infections revealed that costs for MRSA infections were highest with a mean of $35,367 [111]. A large fraction of additional costs that are attributed to the development of a nosocomial infection results from the prolonged hospital stay, rather than from the use of antibiotics to treat these infections [112]. The distribution of cost between hospital stay and costs of antibiotics might be different for infections caused by hVISA or VISA, especially if these infections are treated with relatively expensive antibiotics such as linezolid. A comprehensive economic analysis of factors, which influence the overall cost of antibiotic resistance will be helpful to strengthen the argument that fighting antibiotic resistance is cost-effective [113].

Based on the analysis of published data and on an unpublished Canadian study, it can be estimated that measures to detect and prevent transmission of resistant pathogens will increase hospital costs by $150 to $250 per day [114]. Nevertheless, as has been shown by *Chaix* et al. [115], efforts to control and contain MRSA are cost-effective under most circumstances. Although not yet formally investigated, efforts to prevent the further spread of glycopeptide resistance in *S. aureus* are likely going to be cost-effective as well, as these efforts are essentially similar to the efforts for MRSA control. In order to be successful, it will be important to improve compliance with infection control measures during contact with MRSA infected or colonized patients, as this was found to be poor [116].

Open Questions and Outlook

In order to advance our knowledge about *S. aureus* with glycopeptide resistance and to improve the management of patients infected by such strains, research will be needed in epidemiology, diagnostic microbiology, pathogenesis, and clinical management of glycopeptide resistance.

As reduced susceptibility to vancomycin has been detected in most pandemic MRSA strains, it appears very probable that this type of resistance will become a global problem [117]. Changes in the epidemiology of MRSA may precede changes in the epidemiology of hVISA and VISA. Intensive care units, due to the frequent use of antibiotics and the higher prevalence of patients with nosocomial infections are likely locations for the selection of heteroresistance. The prevalence of MRSA in intensive care units varies markedly, even within countries. A survey of German ICUs found an overall prevalence of MRSA of 14.3% among nosocomial *S. aureus* infections [118]. However, while more than 60% of the surveyed units did not observe any MRSA infection, in some units the MRSA rate was greater than 50%. Clusters and outbreaks were common [118]. A more recent German study, which is based on the surveillance of antibiotic resistance in more than 20,000 isolates that were obtained from patients in 35 ICUs, found methicillin resistance in 19.3% of *S. aureus* [70]. In order to obtain more precise data about the prevalence, clinical impact and management of infections caused by VISA or hVISA, adaptation of current surveillance strategies to include active surveillance for VISA and hVISA in high- risk areas of the hospital should be considered.

As discussed above, detection of VISA and hVISA in clinical samples is a challenge for routine diagnostic laboratories. Etest with a high inoculum appears to be the test with the best performance, while still acceptable regarding workload for diagnostic laboratories. Standardization of recommendations between countries is urgently needed in order to develop common procedures for the management of patients with MRSA, which may possibly exhibit glycopeptide resistance. Once the genetic basis for VISA or hVISA is elucidated, molecular techniques might become available for the diagnosis of this resistance genotype. However, as VISA and hVISA are transient phenotypic states of VSSA, the sensitivity of such mo-

lecular tools might depend on the stability of expression of relevant genes in these strains.

In addition to the determination of molecular markers for the hVISA or VISA phenotype, investigations of the link between *agr* II polymorphism, other regulatory genes, and invasiveness or other microbiological or clinical features of glycopeptide resistant *S. aureus* will be important for the understanding of the pathogenesis of infections caused by such strains.

Finally, more detailed clinical information is needed in order to assess the contribution of glycopeptide-resistance to the clinical course of *S. aureus* infections. Since almost 20% of patients with nasal colonization by MRSA will develop an invasive infection during hospitalization, an increase in the prevalence of MRSA will likely have a negative impact on public health [119]. The invasive potential of VISA or hVISA following colonization of the nares will need to be investigated. MRSA has been recognized as a public health problem in outpatients as well. Several studies have examined the clinical impact of *S. aureus* infections in general, and of MRSA infections in injection drug users in particular [120–122]. Emergence of VISA or hVISA might further complicate the management of infections in this patient population.

Ideally, double-blind, randomized, controlled clinical trials would have to be conducted to determine the approach to the treatment of infections by VISA or hVISA. As these infections are still relatively rare, it appears unlikely that such studies will be realized. However, prospective clinical studies using clear-cut definitions of resistance, predefined clinical endpoints and standardized treatment protocols will be needed in order to gain some insight into the optimal treatment of such infections. In addition, some animal models of infection by glycopeptide-resistant *S. aureus* may be needed in order to determine the role of various treatment strategies. As an example, experimental data, based on models, suggest that lysostaphin or the combination with a beta-lactam antibiotic might be alternatives to consider for the treatment of infections caused by hVISA [123–125].

In conclusion, glycopeptide resistance of *S. aureus* is no longer a theoretical threat. Rather, it is a clinically relevant possibility, which should be considered in every patient with MRSA infection who shows an unfavorable clinical course despite treatment with a glycopeptide. Clinicians should be aware of the pathogenesis, epidemiology, and clinical consequences of glycopeptide resistance in *S. aureus*, since this pathogen remains one of the most important bacteria in modern medicine.

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