First Detection of an Invasive *Staphylococcus aureus* Strain (D958) with Reduced Susceptibility to Glycopeptides in Saudi Arabia

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Strain D958, a methicillin-resistant *Staphylococcus aureus* strain with reduced susceptibility to vancomycin, was isolated from a 69-year-old Saudi male patient presenting with severe sepsis immediately after admission. Despite high serum levels of vancomycin, the same *S. aureus* strain was isolated from five blood culture sets during 1 week. Treatment failure under therapeutic levels of vancomycin prompted us to investigate the resistance profile of this strain in further detail. The MIC values for vancomycin as determined by Etest and microdilution were 3.0 and 2.0 mg/liter, respectively, and remained unchanged during the treatment course. The macro-Etest method showed a MIC of 4 mg/liter. The strain showed liquid vancomycin and lysostaphin MBCs of 2.0 and 5.0 mg/liter, respectively. The isolates were confirmed as heterogeneously vancomycin-intermediate *S. aureus* (hVISA) by vancomycin population analysis profile. The areas under these curves were similar for Mu3 and D958 for vancomycin and teicoplanin (ratio values were 1 and 1.1 for vancomycin and teicoplanin, respectively). Extensive genotyping and molecular characterization demonstrated that the strain harbored a staphylococcal cassette chromosome mec element (SCCmec) type III cassette and was of sequence type ST241, a single-locus variant of the successful multiresistant clone ST239. Microarray results demonstrated that D958 contained numerous resistance determinants (generally plasmid or phage encoded). These results suggest that this strain is constitutively expressing an altered susceptibility to vancomycin. Further studies are warranted to assess the clonal distribution of such strains displaying reduced susceptibility to vancomycin prior to any antimicrobial therapy.

*Staphylococcus aureus* is a major cause of serious hospital- and community-acquired infections associated with morbidity and mortality (42). In recent years, prevalence rates of methicillin-resistant *S. aureus* (MRSA) strains have varied between (and within) countries, but they have increased significantly since the early 1990s. In the first decade of the new millennium, MRSA rates have reached worrisome levels in numerous countries, such as the United Kingdom (40%), France and Greece (35%), and Italy (45%) (53). Simultaneously, the United Kingdom, Ireland, and Greece have reported some of the highest rates of MRSA from bloodstream isolates (44, 41, and 44%, respectively, in 2004) (15). In 1996, the first clinical strain of *S. aureus* with reduced susceptibility to vancomycin (MIC of 8.0 mg/liter) was reported in Japan (25). In 2002, the first two clinical infections caused by vancomycin-resistant *S. aureus* strains (VRSA) were confirmed in the United States (10, 38). The latter report describes the first documented case of an infection caused by VRSA (vancomycin MIC, ≥32 mg/liter) resulting from the transfer of a vanA gene from *Enterococcus faecalis* to *S. aureus*. Although the emergence of vancomycin-intermediate *S. aureus* (VISA, or GISA for glycopeptide-intermediate *S. aureus*) (21, 23) and, most recently, vancomycin-resistant *S. aureus* (10, 38) is an important concern, such cases remain quite rare (seven cases of VRSA in 2002 to 2006 [52]). Nevertheless, vancomycin treatment failures are not uncommon with MRSA infections despite the fact that the organism is apparently fully susceptible (vancomycin MIC, ≥2.0 mg/liter) according to standard methods of in vitro testing (39).

Antimicrobial regimens that provide bactericidal therapy have been demonstrated to be superior to bacteriostatic regimens in the treatment of *S. aureus* bloodstream infections, especially with infective endocarditis (44). Whereas the acquisition of *E. faecalis vanA* was shown to yield to high-level resistance against glycopeptides, the evolution of MRSA to an intermediate level of resistance appears related to multiple factors, including cell wall synthesis and processing (14), autolysis (9), or regulatory events (36). Interestingly, common observations have been reported in the literature for the expression of this “endogenous” resistance to glycopeptides within different genetic backgrounds of closely related MRSA strains. Whereas strains evolving to the GISA phenotype have been observed under glycopeptide therapy (37), other authors reported the spontaneous emergence of such phenomena (58).

As in many areas around the globe, and despite restrictions on prescribing antibiotics, the empirical treatment of *S. aureus* infections according to our policy of antibiotic usage is oxacillin and gentamicin; the use of vancomycin is restricted and limited to suspected or proven MRSA infections. The violation...
of this recommendation cannot be formally excluded in the Kingdom of Saudi Arabia.

An epidemiologic study was performed on MRSA strains (n = 512) isolated between January 2004 and December 2005 from six major hospitals in Riyadh, Saudi Arabia (5, 6). None of the isolates displayed reduced susceptibility to vancomycin, and they yielded MICs in the range of 1.0 to 1.5 mg/liter. Variations observed in the susceptibility of the isolates across different hospitals probably reflect differences in antibiotic usage and thus the development of resistance in these hospitals to different antibiotics (5, 6).

This study reports the first case of MRSA with decreased susceptibility to vancomycin in Saudi Arabia, a strain that resulted in vancomycin treatment failure despite a moderate elevation of MIC to vancomycin.

**RESULTS**

**Case description.** A 69-year-old Saudi male patient known to have chronic heart and kidney failure, hypertension, and asthma presented to our hospital for severe sepsis with fever, abdominal pain, systolic dysfunction, and intracranial hemorrhage. The patient received 1 g of vancomycin and 500 mg/12 h of meropenem as empirical treatment. After 24 h, blood cultures revealed the growth of Gram-positive cocci in clusters that turned out to be *S. aureus* according to the biochemical identification. In addition to penicillin and methicillin, the strain was resistant to ciprofloxacin, levofloxacin, fusidic acid, trimethoprim-sulfamethoxazole, clindamycin, and erythromycin but remained fully susceptible to rifampin, tigecycline, and linezolid. The MIC of vancomycin was <2.0 mg/liter (Dade Behring MicroScan, Sacramento, CA). As hVISA or VISA isolates are not reliably detected by automated methods, Etest and microdilution assays were performed and yielded MICs of 3.0 and 2.0 mg/liter, respectively; finally, the macro-Etest showed a MIC of 4 mg/liter. Note that in addition, strain D958 showed a particularly high level of resistance to lysisostaphin (MIC, 5.0 mg/liter), two times higher than that observed for Mu50. Vancomycin MIC values obtained from the Etest and the microdilution method were 3.0 and 2.0 mg/liter, respectively. The MIC of teicoplanin was 1 mg/liter. The MBC was assessed at 2 mg/liter for vancomycin and 1 mg/liter for teicoplanin.

Population analysis showed that strain D958 displays a Mu3-like profile, with a few colonies still growing at 8 mg/liter of vancomycin or teicoplanin (Fig. 1). The areas under the curves were similar for Mu3 and D958 compared to those for the fully susceptible isolate for vancomycin (7-fold and 5-fold higher, whereas Mu50 showed a 29-fold higher area) and for teicoplanin (7-fold and 8-fold higher, whereas Mu50 showed a 29-fold higher area). The patient was then switched to receiving vancomycin (500 mg/12 h) and gentamicin (80 mg/24 h). During 1 week of combined antimicrobial therapy (with vancomycin trough concentrations always greater than 15 mg/liter), high fever and leukocytosis persisted and the same MRSA strain was recovered from five separate blood culture sets obtained during the following days. Treatment failure prompted us to replace vancomycin with linezolid (600 mg/12 h). After 48 h of linezolid treatment, the patient became afebrile, white blood cells counts decreased, and blood cultures became negative.

**Molecular characterization of strain D958.** We first performed a multiple locus variable-number tandem repeat analysis (MLVA) (20) and compared the obtained profile with those of control strains. Figure 2A shows that strain D958 is not clonal with any strain from the U.S. collection. However, D958 clusters quite closely to the reference strain of ST239. This observation was confirmed by MLST that showed that strain D958 harbored sequence type ST241 and appeared thus not clonal with any strain from the U.S. collection. However, D958 clusters quite closely to the reference strain of ST239.

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This observation was confirmed by MLST that showed that strain D958 harbored sequence type ST241 and appeared thus indeed related to ST239 (Fig. 2B). ST241 is a single-locus variant of ST239, both belonging to clonal complex 8 (CC8) and showing the profiles 2.3.1.1.4.30 and 2.3.1.1.4.3, respectively. Figure 2B includes isolates with sequence types previously reported as GISA. The ST239 strain was previously identified by our group (58) and more recently characterized as a genetic background that can evolve to a GISA phenotype (26, 51). This strain has been extensively documented, including the
recent pandemic clone in many Asian countries (Taiwan, Thailand, China) but also in Germany (17, 62). To date, GISA strains have mainly been reported in CC5 (34, 41) but recent results suggest that their precursor hVISA isolates exist in the five most frequent clonal complexes found in human medicine (28).

Complete genome hybridization experiments were performed to evaluate the relatedness of strain D958 with characterized clinical isolates, in relation to its genomic content. Microarrays revealed that D958 segregates closely with other CC8 strains and more distantly to the other clonal complexes tested, such as CC1 or CC5 (Fig. 2C). D958 contains numerous antimicrobial resistance determinants, such as mecA, as well as norA and the fosfomycin resistance gene fosB, but neither the kanamycin nucleotidyltransferase (aadD) nor the tetracycline resistance element were recovered, as predicted by the susceptibility profile. In terms of putative virulence factors, strain D958 harbors the two clumping factors (clfA and clfB), a complete ica locus, and the fibronectin-binding proteins as well as alpha, beta, and gamma hemolysins, but it contains only a partial cap operon encoding the capsule. Finally, D958 contains the enterotoxin gene sea, which is described as a potential marker of virulence for strains involved in severe infections (18, 57).

DISCUSSION

Glycopeptides remain the recommended therapy for patients with methicillin-resistant Staphylococcus aureus infections. Treatment failure is common, with 10 to 20% in cases of endocarditis and 40% in lower respiratory tract infections (7, 40). Causes for treatment failures are not fully understood: clinical failures have been reported in patients infected with VISA (2, 43) or hVISA (4, 60, 64) strains. More recently, treatment failure has also been reported with strains displaying MICs that were within the susceptibility range (≤2.0 mg/liter) (50). Also, a number of small studies and case reports documented poor clinical response to vancomycin therapy in bacteremia and/or endocarditis caused by vancomycin-tolerant strains of S. aureus and the need to use additional agents to produce a bactericidal effect (27, 45).

After the initial description of MRSA strains with decreased susceptibility to glycopeptides in Japan (25), clinical isolates showing similar phenotypes were repeatedly reported in various countries (8, 31, 56), exhibiting also decreased susceptibilities to glycopeptides (glycopeptide-intermediate S. aureus [GISA]). These strains represent a crucial challenge for antimicrobial therapy, antimicrobial susceptibility testing, and hospital infection control. This context is worrisome, as a trend of increased MIC levels against glycopeptides was observed in various hospitals (59). Methods that typically detect hVISA or VISA are nonautomated MIC methods, including reference broth microdilution, agar dilution, and Etest using a 0.5 McFarland standard. In our case, MIC as determined by the automated system (MicroScan) was <2.0 mg/liter. However, considering the MIC values observed for strain D958, experts recommended performing population analysis (33, 55), which showed that D958 displays a Mu3-like profile with a few colonies still present in the presence of 8 mg/liter of vancomycin. Since the first Japanese report 10 years ago, VISA strains have been reported throughout the world, but they remain rather uncommon, with about only 100 such isolates reported so far (3). Approximately 90% of these strains show heterogeneous resistance (hVISA), which is also the case for strain D958 described in this study, whereas 10% are homogeneously resistant (VISA) (28). In contrast to the reports of Hiramatsu et al. (24, 25), a study carried out by Ike et al. found no VISA or hVISA strains among more than 6,000 MRSA strains originating from nearly 300 Japanese hospitals (30). Similarly, an analysis of more than 600 MRSA strains from 33 U.S. hospitals in 1997 failed to identify any homogeneous VISA strains and disclosed only two hVISA strains (29). A survey in 1998–1999 of 303 epidemic MRSA (EMRSA), EMRSA-15, and EMRSA-16 isolates from more than 50 hospitals in England and Wales found no homogeneous VISA and reported only one hVISA strain (63). Several studies showed that the surveillance of hVISA was not performed routinely and that the methods used differed between laboratories, thus preventing

FIG. 1. Population analysis of MRSA strain D958 in the presence of vancomycin or teicoplanin. Growth of subpopulations of D958 was still observed at 8.0 mg/liter of either vancomycin or teicoplanin. For comparison purposes, strains SA5454, Mu3 and Mu50, representative of fully susceptible, hVISA, and VISA phenotypes, respectively, are shown in the figure.
accurate interpretation of the actual hVISA prevalence (35). Currently, hVISA isolates are more frequently reported than previously thought. In a recent prospective study performed in France, the prevalence of hVISA was 11% (22), which is similar to previous reports (47, 54) from other geographic locations. In Saudi Arabia, hVISA infection was never reported in the past, and its clinical significance appears therefore difficult to assess. However, clinical failure with vancomycin has been already observed. As the Etest macromethod appears an efficient method for hVISA detection, and it is neither labor-intensive nor too costly, its utilization for the routine detection of such events appears now in the official recommendation.

The VISA strains are distinct from high-level glycopeptide-resistant strains (VRSA) that result from the acquisition of the \textit{vanA} gene from \textit{Enterococcus faecalis} (61). In this study, however, strain D958 was discovered due to vancomycin treatment failure. In addition to a decreased susceptibility to vancomycin, strain D958 showed a particularly low susceptibility level (5

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**FIG. 2.** Genotyping and relatedness of D958 with control isolates. MLVA (A), MLST (B), and microarray comparative genomic hybridization (CGH) (C) trees of strain D958 compared to reference isolates. For reproducibility purposes, a duplicate from two independent DNA extractions of strain D958 is shown on the MLVA tree. For microarray analysis, background-subtracted data were expressed as log_{10} ratios and analyzed by two-way clustering. Probes yielding positive and negative signals are shown in blue and yellow, respectively. Strain D958 shows relatedness using all three methods with isolates already described as GISA, such as ST239 (26, 51).
mg/liter) to the muropeptide hydrolytic enzyme lysostaphin, which is also the case for strain Mu50 showing a MBC of 2.5 mg/liter, suggesting potential alterations in the cell wall structure, a bacterial envelope previously shown to be involved in the resistance mechanism for VISA strains. Strain D958 appears to be related to ST239 and has potentially evolved to an hVISA phenotype within our hospital in Riyadh. Previous work suggests that exposure or nonoptimal utilization of vancomycin could yield to the emergence of hVISA or VISA strains, at least within certain genotypes of MRSA. For example, Sakoulas et al. have determined in vitro that the emergence of hVISA or VISA may occur when *S. aureus* strains with a downregulated or defective *agr* locus are exposed to suboptimal vancomycin concentrations (48) or simply exposed for prolonged periods of time in the context of recurrent bacteremia (49). In the Kingdom of Saudi Arabia, Al-Mustafa et al. have demonstrated that 29 antimicrobial agents were identified as being available for poultry use, of which 22 (75.9%) were important for the treatment of human infections (enrofloxacin, oxytetracycline, doxycycline, ampicillin, neomycin, sulfamethoxazole, colistin, and erythromycin were the most frequently used drugs) (1). According to the authors, the use of antimicrobial agents in food-producing animals in Saudi Arabia has become an important public health threat and may complicate the treatment of human infections.

In conclusion, vancomycin treatment failure is not uncommon when treating *S. aureus* bacteremia or endocarditis. In such cases, automated MIC and disk diffusion methods are not reliable and broth microdilution as well as Etest methods should be applied to detect potential (h)VISA strains. Screening for heterogeneous and low-level resistance to vancomycin of MRSA strains is essential to evaluate their incidence and the frequency of emergence of hVISA or VISA subclones following vancomycin administration. This systematic screening may soon become a necessary part of infection control practices when glycopeptides are used. Finally, the detection of strain D958 should prompt vigorous infection control measures to prevent the spread of this clone.

REFERENCES


