Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an opportunistic pathogen, and the glycopeptide antibiotic vancomycin is generally the first choice of treatment for severe MRSA infections. Vancomycin has been used for over 5 decades, until 1997 when reduced vancomycin susceptibility in clinical MRSA isolate was first reported and, strains with various degrees of vancomycin resistance identified and classified as: high-level resistance, vancomycin intermediate and heteroresistant *S. aureus* (hVISA), which are becoming ever more prevalent. Therefore, remember and study the evolution of resistance profile will allow the scientific community to better understand the path taken by this microorganism. Once the use of this antimicrobial treatment was repeatedly reported as hVISA selector, which is associated to vancomycin treatment failure. With it, the expectations of new studies have focused on new antimicrobial drugs and mechanisms to prevent the spread of existing pathogens.

Keywords

MRSA; Vancomycin; hVISA; Treatment failure.

Introduction

*Staphylococcus aureus* is a versatile organism that causes important bacterial infections, and generally acts as an opportunistic agent to colonize skin and mucous membranes [1]. Its main mode of transmission is by direct contact with colonized or infected individuals and objects or surfaces contaminated [2]. Thus, in hospital settings infections are primarily transmitted by health-care workers hands transiently contaminated after contact with patient carrying the microorganism [3].

Its antimicrobial resistance was reported shortly after the introduction of penicillin in 1941. Two years later, 50% of worldwide hospital samples already showed resistant [4, 5] and by the 1970s about 70 to 85% prevalence of penicillinase-producing strains was found in United States, this proportion, which soon increased to 80-90% [4]. In Brazil, approximately 93% of *S. aureus* isolates are penicillin-resistant [6].

Penicillin-resistant *S. aureus*, first limited to health-care settings, have become, over time prevalent in community, and thus, methicillin and other penicillinase-resistant penicillins have been developed for these infections treatment successfully. However, methicillin-resistant *S. aureus* (MRSA) emerged and spread, initially within the hospital and then in community, in parallel to penicillin-resistance [5].

Methicillin-resistance mechanism in *S. aureus* is related to production of a low-affinity penicillin binding protein known as PBP2a or PBP2’, which is encoded by mecA gene [3]. This is carried out by a mobile genetic element ("Staphylococcal Cassette Chromosome mec" SCCmec) which confers resistance to all penicillins, cephalosporins and carbapenems [7, 8]. Moreover, usually transport other resistance genes to other classes of antimicrobial agents, including macrolides, fluoroquinolones, clindamycin and rifampin, resulting in multidrug resistance of samples [9].

Staphylococcus aureus and vancomycin resistance: what does it matter?
Turlej, Hryniewicz and Empel (2011) [8] described the already identified SCCmec types I to XI, warning for the difficulty of establishing a naming worldwide convergence. This way the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements was created to standardize SCCmec naming system.

Currently there are two MRSA phenotypes: hospital-acquired (HA-MRSA) and community-acquired (CA-MRSA). HA-MRSA as well as widespread throughout the world is responsible for at least one-third of all S. aureus infections, with an estimated mortality rate of 6.3 per 100,000 individuals. The risk for HA-MRSA infections is related to: chronic diseases, dialysis, malignancy, prolonged exposure to antimicrobial agents, especially cephalosporin, aminoglycosides and fluoroquinolones, age, insulin dependent diabetes, smoking, obesity, dermatitis and prolonged hospital stay [10].

At the end of 1990s MRSA infections began to be observed in the community in healthy individuals without traditional risk factors for acquisition of MRSA infections. The phenotype responsible for such occurrences, CA-MRSA has emerged as a new pathogen which diverges from the hospital phenotype not only genetically but also epidemiologically [11].

The genetic divergence between HA-MRSA and CA-MRSA is expressed mainly in SCCmec type carried by the microorganism [9]. SCCmec types I, II, III can be found predominantly in HA-MRSA, while IV and V are found in CA-MRSA [8]. SCCmec type IV is small (20.9 to 24.3 kb) and show no additional resistance genes to non-β-lactam antimicrobial [8, 12]. SCCmec IV or V with lukF-PV genes, which encodes for Panton-Valentine leukocidin (PVL) production, a toxin that destroys human leukocytes and cause tissue damage, are CA-MRSA genetic markers [8].

In contrast to HA-MRSA, for which the risk factors are well established, CA-MRSA can occur in healthy individuals suggesting that these have higher virulence than traditional HA-MRSA samples. Moreover, CA-MRSA strains as USA300, is capable of rapid spread, which might explain the worldwide dissemination [13].

MRSA spread was followed by the emergence of other antimicrobial resistance as aminoglycosides, quinolones, macrolides and tetracycline [5]. In 1996 the first clinical vancomycin-intermediate Staphylococcus aureus (VISA) strain (Mu50) with a vancomycin minimum inhibitory concentration (MIC) of 8 µg/mL and the hetero-VISA (hVISA) strain (Mu3) with an MIC of 2 µg/mL were isolated, from a surgical site infection in a 4 month-old male infant, for which the use of vancomycin was not effective [14]. Recently, the concern spent the focus on emergence and dissemination of vancomycin resistance (VRSA) [15].

Clinical and Laboratory Standards Institute (CLSI) defined vancomycin MIC values to S. aureus as follows: VSSA (Vancomycin-sensitive S. aureus) MIC ≤ 2 µg/mL, VISA (Vancomycin-intermediate S. aureus) 4 ≥ MIC ≤ 8 µg/mL and VRSA (Vancomycin-resistant S. aureus) MIC ≥ 16 µg/mL [7]. Furthermore, hVISA (Heteroresistant VISA), with MIC of 4 µg/mL when tested by routine methods, but having subpopulations with a higher MIC in the order of 106 cells [15, 16].

Several tests can be used for VISA detection, however, there is still no standard method for accurately detecting hVISA [17]. This fact is explained because the resistant subpopulation can be present in a low ratio compared to the susceptible population (10-5 and 10-6). Among the techniques for screening can be used: Etest, broth microdilution, Etest GRD and Simplified Population Analysis Profile (PAP) [7]. Currently, the “gold standard” for hVISA confirmation is the population analysis profile/area under the curve (PAP/AUC) which detects hVISA samples with vancomycin MICs as low as 0.5 to 1 µg/mL [7].

hVISA infections are associated with frequent failures in glycopeptides therapy and infectious recurrence reflecting the microbial resistance characteristics. According to Hiramatsu et al. al. (2014) [18], hVISA strains are composed of resistant subpopulations with various degrees of vancomycin resistance and, during the course of infection treated by this antimicrobial, most of the cell population are depressed, especially those with MIC lower than 4 µg/mL compatible with clinical improvement observed initially. However, the small numbers of VISA that survives to vancomycin therapy multiply and cause infection exacerbation. This way susceptibility tests aims to predict the response to a given drug in the context of the infection. Howden (2010) [7] reflects that studies aiming to correlate the presence of hVISA with the progress of infection.
glycopeptides treatment must determine if clinical sample was obtained before or after treatment failure.

After the first reports of VISA and hVISA in Japan did not take long for this phenotype was recognized around the world [19]. hVISA samples have been reported in the United States, Europe, Asia, Oceania and South America. The world observed variation in hVISA frequency is partly due to differences in laboratory settings, and on the other hand, the methodology used by prospective studies [7]. Additionally, resistant isolates can convert to susceptible phenotype, as exemplified by Howden and colleagues (2010) [7] in which four strains VISA (NI, IM, PC and Mu50), have suffered for a susceptible phenotype reversion after 15 days passage on non-selective medium. Among the four strains studied three maintained a subpopulation which subsequently grew at 4 µg/mL of vancomycin. Many strains with MIC for 8 µg/mL of vancomycin have been reported throughout the world and associated with patients during therapy with the drug, and MICs demonstrated that as low as 2 µg/mL when tested again demonstrating the instability of this resistance phenotype, with a tendency to reduction in MICs when the selection pressure of vancomycin is removed [20].

The analysis of 4210 *S. aureus* isolates obtained from 43 medical centers in the US, detected by Etest GRD for screening and PAP-AUC for confirmation showed a rate of 16% of hVISA [15]. In a study conducted in Australia, Van Hal and colleagues (2011) [21] found 12% of hVISA between 458 samples of MRSA isolated from bloodstream infection. Although the emergence of VISA is considered a result of the extensive use of glycopeptides, Katayama and colleagues (2009) [22] consider that hVISA may be associated with frequent exposure to MRSA on imipenem.

Although predominantly reported for MRSA, the hVISA phenotype has been described in strains of methicillin-sensitive *S. aureus* (MSSA) [7]. In a review by Liu and Chambers 2003 [17], in 14 studies evaluated, hVISA rates were 2.16% in samples of MRSA and 0.05% in MSSA samples.

Despite the uncertainties about the role of genes in increased resistance to vancomycin, some studies using electronic microscopy revealed that all clinical samples VISA had a common feature, thick cell walls. The thickening of the cell wall without vancomycin may not be obvious by electronic microscopy, but after exposure to vancomycin, becomes more evident, this thickening prevents the spread of vancomycin to its active site in the cell wall [23].

The concern about the acquisition of the vanA gene in isolates of *S. aureus* came up with the emergence of vancomycin-resistant Enterococcus faecium in 1980 [24]. Howden and colleagues (2014) [25] stated that although the genetic determinants in hVISA and VISA are less understood DNA sequencing has allowed the genetic comparison of isogenics pairs hVISA and VISA and showed that, as opposed to what occurs with MRSA and VRSA resistance to vancomycin these phenotypes is not related to the acquisition of foreign genes. While for VRSA resistance is attributed to the acquisition of vanA carried by transposon T1546 [26]) in VISA and hVISA mutations in regulatory genes of *S. aureus* have changed their functions, completely or partially. Mutations occur in a large number of genetic loci expressing pleiotropic effects that include differences in vancomycin resistance level between isolates [25]. A large number of genes appears to be involved in the production of the precursors of staphylococcal cell wall [18]. Relevant genes include the femA, femB, and femC femX, genes encoding the penicillin-binding proteins (PBP) (pbpA pbpB, pbpC and pbpD) and regulatory genes involved in cell wall biosynthesis, such as vraSR [23].

These regulatory genes involved in cell wall synthesis and bacterial metabolism seem to be the targets of various single mutations carried by isolated hVISA and VISA [23], each of promoting specific impact on the accumulation of resistance [25]. In other cases a single mutation is sufficient for resistance expression [7], but certainly not at the same level when compared to those that accumulate multiple mutations [25]. In review presented by Howden and colleagues (2014) [25] the main genes involved in genetic determination of VISA and hVISA are walKR, vraRS and graRS, good as the gene coding for the production of RNA polymerase B subunit (rpoB). In a study of 33 clinical isolates of VISA, 14 (42.4%) had mutations in at least one of the genes vraT, vraS and vraR; 18 (54.5%) in walK and 21 (63.6%) in rpoB. Another observation was the mutation occurred in 29 of the 33 isolates (87.9%) in any of the studied gene loci: walK, vraRS and rpoB [23]. The high number of alternative mutations with potential to convert hVISA in VISA may account the high frequency of emergency VISA from hVISA [23].
the reversal of phenomenon can occur in the VISA to hVISA, VISA to VSSA and hVISA to VSSA. Although the genetic mechanisms of these events have not yet been well elucidated, the findings show a strong pressure to reverse the resistance phenotype when selective removal pressure represented by the presence of the antimicrobial, and has a significance of greater energetic cost during the expression of the VISA phenotype [25]. The account of vancomycin resistance emergence, drug of choice for treatment of hVISA, associated to high rates of hospital infections by that microorganism become indispensable to the adoption of control measures for this pathogen in hospital environments [5]. Preventive procedures such as isolation of positive cases of hVISA, rational antimicrobial use, control of carriers and education measures, as well as constantly surveillance this microorganism, especially in Intensive Care Units [27] and the need to elucidate susceptibility to vancomycin MRSA clinical samples are correlated with the corresponding results in clinical patients. Therefore, remember and study the evolution of resistance profile will allow the scientific community to better understand the path taken by this microorganism.

References


