Antimicrobial resistance in *Staphylococcus* spp.

M. L. Ribeiro de Souza da Cunha, and D. R. Ustulin

UNESP, Department of Microbiology and Immunology, Bioscience Institute, Univ. Estadual Paulista, Ruião Júnior, 18618-970, Botucatu, SP, Brazil

*Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) are the main agents of nosocomial infections. The antimicrobial resistance of these microorganisms has increased worldwide, justified by the selective pressure caused by the use of broad spectrum antibiotics. The prophylactic antibiotherapy is also a concerning factor, because it increases the risk of infection, and difficult its management. Most staphylococci are resistant to penicillin, methicillin and actually reports of vancomycin resistance are found in the literature, and the resistance mechanisms are identical in *S. aureus* and CoNS. Detection of oxacillin resistant staphylococci is important to guide therapies and also to avoid use of vancomycin, which is an antimicrobial agent with therapeutic complications, and can lead to selection of resistant strains. This review aims to describe important points of the resistance to antibiotics in *Staphylococcus* spp., regarding the therapies and laboratorial aspects.

**Keywords** *Staphylococcus* spp.; resistance

1. Introduction

Members of the *Staphylococcus* genus are Gram-positive cocci measuring 0.5 to 1.5 μm in diameter. They appear isolated or in irregular grape-like clusters which are motionless, non-spore forming and catalase positive. Also, most species are facultative anaerobic [1].

The *Staphylococcus* genus comprises 45 species, most of which are coagulase-negative, and coagulase is exclusively synthesized by *S. aureus*, *S. schleiferi* subsp. *coagulans*, *S. intermedius*, *S. pseudointermedius*, *S. hyicus*, *S. delphini* and *S. lutrae* while other species are referred to as coagulase-negative staphylococci (CoNS) [2]. These microorganisms are broadly distributed in nature and particularly found in the skin and mucosal membranes of mammals and in other animals. Some *Staphylococcus* species show a preference for certain habitats in their hosts. *S. aureus* prefers the nostrils, especially in adult humans, and some CoNS species, such as *S. capitis*, are found in specific niches, namely the head. *S. auricularis* is found in the auditory canal, and *S. saprophyticus* in the genital and urinary tract [1].

Staphylococci usually maintain a benign or symbiotic relationship with their hosts. However, if the cutaneous barrier is broken by trauma or by the presence foreign articles, these microorganisms may reach other tissues and proliferate, thus developing a pathogenic behavior [1].

*S. aureus* is the most important species and the agents of a variety of infections. Nosocomial infections caused by such microorganisms have been the major causes of morbidity and mortality, and they are currently among the ten main causes of death worldwide and the basic cause of death in 1% of cases [3].

CoNS were only acknowledged as important pathogens in nosocomial infections as from the 1970s. Until then they were considered as simple contaminants of biological samples and given little attention. In the last few years, they have been described as a frequent cause of nosocomial infections and cited as the third most common agents in nosocomial blood infections [1]. This is due to advancement in medicine and in its invasive technological resources, the world population’s increased life span, the larger number of debilitating diseases, neoplasias and immunodeficiency, which have made humans vulnerable hosts to CoNS [1,3].

The infections caused by staphylococci are frequently acute and pyrogenic and, if untreated, they may cause bacteremia involving various organs. The most common infections include furuncles, cellulitis, impetigo and surgical wound sites. Some of the most serious infections are bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, meningitis and abscesses in the muscles, genital and urinary tract, central nervous system and various intra-abdominal organs [4,5].

*S. aureus* produces a large variety of toxins that can be divided into two groups: lysis-inducing toxins, which are referred to as hemolysins and can produce lesion directly in the external membrane of target cells [6], and those referred to as superantigens toxins, which do not have direct lytic action, but can produce lesion through the overproduction of cytokines by T cells and activated macrophages [7, 8]. Four cytolitic toxins are produced, including the alpha (α), beta (β), delta (δ) and gamma (γ) toxins. Such toxins have also been described as hemolysins based on their capacity to lyse red blood cells, but, since their biological activity is not limited to such cell type, the term cytolitic toxin has been introduced [9]. Staphylococcal toxins, referred to as superantigens, are antigenically classified as toxin 1 of the toxic shock syndrome (TSST-1) and as enterotoxins. Through the production of such superantigens, a series of toxic effects is triggered, such as the toxic shock syndrome, food poisoning and various disturbances of the immune system [7, 8].

The pathogenicity of the species is due to both the absorption of these pre-formed toxins and the powerful invasive capacity that leads to bacteremia and abscesses disseminated in all organs. From any focuses, these microorganisms
can disseminate through the lymphatic vessels and the blood stream to other parts of the body [1]. Some host-related factors predispose to infections by these germs, among which are age, base chronic diseases, intravenous catheter use, or behavioral factors, such as the use of intravenous drugs by drug addicts and long hospitalizations [1, 5]. Infection and propagation by contact take greater importance in hospitals, where a large number of staff members and patients host resistant strains in their noses and skin [1]. The prevalence of these drug-resistant germs, particularly in hospitals, is explained by the selective pressure caused by the use of broad-spectrum antibiotics, the lack of a therapeutic conduct aiming at the use of antibiotics with an action spectrum directed only to etiological agents. The improper prophylactic use of antibiotics that can select resistant microorganisms also contributes to this situation, thus increasing not only risks for infection, but also its severity and difficulty of treatment [1, 5].

Antimicrobial resistance in these microorganisms is easily acquired, and the high transmissibility of plasmids among strains in hospitals and the abusive use of antimicrobial drugs have been important factors related to the transfer of resistance genes and to the selection of multiresistant strains [10]. At times, alteration in pathogen virulence follows resistance expression, thus resulting in the dissemination of such multiresistant pathogenic strains among hospitalized patients. Studies based on DNA analysis suggest the cross-transmission of strains within hospitals and their permanence in the hospital environment for long periods of time [11].

As CoNS infection has been increasingly characterized, the interest in studying its susceptibility to various antimicrobials has also proportionally increased. According to Archer and Climo [12], there is an association between the increased percent frequency of CoNS in the etiology of nosocomial bacteremia and these microorganisms’ resistance to antimicrobial agents. The lineages isolated from clinical specimens have shown to be frequently resistant to the antibiotic commonly used in hospitals, as reported by Livermore [13].

When investigating the incidence of multiresistance in S. epidermidis, which is defined as resistance to penicillin or methicillin combined with resistance to one or more aminoglycosides, Möller [14] observed that, from 1981 to 1985, the incidence of multiresistant lineages almost doubled. In our environment, a study conducted by Cunha and Lopes [15] on CoNS isolated from newborns showed 50% resistance to oxacillin. More recently, in a study by Cunha et al. [16] on CoNS isolated from patients from the Botucatu School of Medicine University Hospital, the authors observed an 82.5% rate of oxacillin resistance. The mechanisms responsible for resistance in CoNS are identical to those in S. aureus; nevertheless, resistance mediated by the mecA gene is frequently expressed in lower levels than in that in methicillin-resistant Staphylococcus aureus (MRSA), thus making detection even more difficult [13].

The detection of oxacillin resistance in staphylococci is important to guide therapy and to prevent patients from being unnecessarily treated with vancomycin, which is an antimicrobial that poses therapeutic complications and may lead to the selection of resistant strains [17]. The prevalence rates of MRSA and methicillin-resistant coagulase-negative Staphylococcus (MRCoNS) vary a great deal, particularly in function of the institution’s type and size.

The emergence of antimicrobial resistance to drugs thus reflects a serious problem related to a significant increase in clinical antimicrobial therapy in patients affected by staphylococcal infections, particularly when involving microorganisms with numerous virulence factors and genetic mechanisms for the acquisition of such characteristic in the hospital environment. Laboratory recognition of such resistance and its clinical implications has become increasingly more relevant, with care-related and epidemiological impact. This review study aims at describing important aspects concerning resistance in Staphylococcus spp. that must be considered from the clinical and laboratory viewpoints.

2. Penicillin resistance

In the pre-antibiotic era, the treatment prognosis for severe staphylococcal infection was extremely poor. The introduction of penicillin in therapeutics in 1944 temporarily solved the problem of such infections. However, in 1946, the first resistant samples were found, with 6% of S. aureus producing penicillinase. In 1948, more than 50% of the S. aureus samples from hospitals were penicillin resistant [18]. Such proportion subsequently increased to approximately 80 to 90% [19]. The progressive dissemination of such samples drastically reduced the therapeutic value of that antimicrobial, and currently, only a small percentage of S. aureus samples are sensitive. Similar data are observed in Brazil and, according to data from the Martins et al. [20] of the Botucatu School of Medicine (FMB) University Hospital (HC), 93% of the S. aureus samples are also resistant to that drug.

CoNS also show high resistance to penicillin of approximately 70%, as reported in a study conducted by Tavares [21] and according to a study performed by Cunha et al. [16] on CoNS isolates from patients from the Botucatu School of Medicine University Hospital, the production of enzyme penicillinase was observed in 89.3% of the studied samples. Penicillin resistance is attributed to the production of enzymes that are capable of inhibiting drug action. They are referred to as penicillinas, or more generally as β-lactamases, and they can hydrolyze the β-lactam ring of penicillin [1]. Penicillinase production may be plasmid-mediated, but the chromosomal integration of that gene is frequent [13].

Other antibiotics were developed soon after penicillin introduction, such as cloramphenicol, erythromycin, streptomycin and tetracycline. Initially, all of them were active against S. aureus, but resistance emerged, and it was frequently mediated by plasmids and transposons [22]. A resistance profile study on 626 S. aureus samples isolated
from 25 Brazilian hospitals showed resistance percentages of 29.7%, 47.6% and 47.9% to cloramphenicol, tetracycline and erythromycin, respectively [23].

3. Methicillin resistance

The use of methicillin and other semi-synthetic penicillin types, such as oxacillin and penicillinase-resistant methicillin, which began in 1959, represented a significant phase in anti-staphylococcal therapeutics. However, resistance to these drugs was detected approximately two years later [24]. Methicillin-resistant strains rapidly spread and their frequency has increased in several geographic regions, particularly leading to nosocomial infection outbreaks [13]. When resistance was firstly described in 1961, methicillin was used in sensitivity tests and in the treatment of infections caused by *S. aureus*. However, in the early 1990s, oxacillin, which belongs to the same class of drugs, was selected as the chosen agent for treatment and sensitivity tests, and the acronym MRSA (Methicillin-Resistant *Staphylococcus aureus*) is still used to describe resistance due to its historical role [19].

Intrinsic oxacillin resistance in *S. aureus* is mediated by the production of a supplementary (PBP 2′ or PBP 2a) penicillin-binding protein (PBP) that shows low affinity with semi-synthetic penicillin. The genetic determinant of that protein has a chromosomal nature, the *mecA* gene. Such gene is identical in all staphylococcus lineages and, therefore, a useful molecular marker for oxacillin resistance [25].

To destroy bacteria, many antibiotics bind to PBP so as to make them inactive. These proteins are involved in the construction of the microorganisms’ cellular wall and, without such well-formed wall, bacteria cannot maintain their integrity and die. While staphylococcal strains normally use three penicillin-binding proteins, PBPs 1, 2 and 3, in their cellular wall synthesis, methicillin- or oxacillin-resistant staphylococci (MRSA) have a supplementary PBP, PBP 2′ or PBP 2a. Therefore, when the *mecA* gene is present, the cell can grow in the presence of oxacillin and of other β-lactams [13].

Although resistance mediated by the *mecA* gene is present in all cells of the population with intrinsic resistance, it can only be expressed by a small percentage of such cells, thus leading to the so-called heterogeneous resistance. Resistance expression in lineages with intrinsic resistance has been categorized into four phenotypic classes; classes 1 to 4, in which class 1 is the most heterogeneous and class 4 is the homogeneous one [26]. The majority of cells (99.9 or 99.99%) in the culture of lineages with class-1 heterogeneous resistance show minimum inhibitory concentration (MIC) of 1.5 to 3 µg/ml, but such culture also contains a small number of bacteria (10⁻⁷ to 10⁻⁸) that could form colonies even in the presence of 25 µg/ml or more of oxacillin (Table 1). In class-2 lineage cultures, the majority of cells (≥ 99.9%) show MIC of 6 to 12 µg/ml, and in these cultures, the frequency of highly resistant cells (capable of growing in the presence of 25 µg/ml) is higher (10⁻³) than in class-1 lineages [26]. Class-3 lineage cultures consist of bacteria (99 to 99.9%) that show high levels of oxacillin resistance (MIC = 50 to 200 µg/ml), but they usually have a subpopulation (10⁻³) of highly resistant cells that are capable of forming colonies even in the presence of 300 to 400 µg de oxacillin/ml. Class-4 cultures comprise cells with homogeneous resistance, with all cells showing high resistance levels and MIC of 400 to 1,000 µg/ml [26].

Other resistance modalities have also been described in lineages that do not show the *mecA* gene, and these are referred to as borderline. Borderline resistance results from two mechanisms, the first of which would be oxacillin inactivation mediated by the hyperproduction of β-lactamase [27], and the second is modified resistance, referred to as MOD-SA, which is mediated by alteration of intrinsic PBPs with affinity for altered oxacillin [28]. These resistance modalities show low resistance levels, MIC of 8 µg/ml [29].

The phenotypic expression codified by the *mecA* gene is affected by various factors, including pH, temperature and osmolarity [29]. When proper conditions are used for laboratory MRSA detection, including Mueller-Hinton agar supplementation with NaCl and adequate temperature and time, as recommended by CLSI (Clinical and Laboratory Standards Institute), detection is achieved without much difficulty. However, for more heterogeneous lineages, detection can be more difficult, even with reference methods [29].

Adequate detection of oxacillin resistance mediated by the *mecA* gene is important for clinical laboratories. Although the recommended methods detect most of the oxacillin-resistant lineages, there are two situations that require additional phases to confirm sensitivity or resistance. The first is the occurrence of extremely heterogeneous lineages that are found to be sensitive by reference methods. The second is the occurrence of borderline resistance (MIC close to the sensitivity breakpoint), which must be differentiated from resistance mediated by the *mecA* gene as long as the clinical significance of the resistance determined by the *mecA* gene is greater. Experimental studies on animals and some clinical data have shown that the use of β-lactam antibiotics was effective in infections caused by lineages without the *mecA* gene and with low resistance levels (borderline) [30, 31]. Nevertheless, infections caused by isolates with the *mecA* gene require vancomycin treatment [32].
**Table 1** Modalities of oxacillin resistance.

<table>
<thead>
<tr>
<th>Phenotypic Expression</th>
<th>Borderline</th>
<th>MOD-SA</th>
<th>Intrinsic Class 1</th>
<th>Intrinsic Class 2</th>
<th>Intrinsic Class 3</th>
<th>Intrinsic Class 4</th>
<th>Homogeneous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>mecA</strong></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>PBP 2a</strong></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td><strong>β-lactamase</strong></td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td><strong>MIC (µg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Majority</td>
<td>4-8</td>
<td>4-8</td>
<td>1.5-3</td>
<td>6-12</td>
<td>50-200</td>
<td>400-1,000</td>
<td></td>
</tr>
<tr>
<td>Subpopulation</td>
<td>-</td>
<td>-</td>
<td>25-100</td>
<td>25-100</td>
<td>300-400</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Subpopulation Frequency</td>
<td>-</td>
<td>-</td>
<td>$10^2$</td>
<td>$10^5$</td>
<td>$10^3$</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Source: Tomacz et al. [26]

The reference methods recommended by NCCLS or CLSI for detecting oxacillin resistance in *S. aureus* include MIC determination by the method of drug dilution in agar or broth, the disk-diffusion method, the Mueller-Hinton (MH) agar screening method added with 4% of NaCl and 6 µg of oxacillin and, more recently, cefoxitin disk diffusion testing [19, 33].

In dilution methods, the sensitivity to detect isolates with resistance mediated by the *mecA* gene varies from 98 to 100% [34]. Although few studies report the heterogeneity level of tested samples, it is supposed that resistant lineages undetected by dilution methods would be more heterogeneous lineages [29].

Studies evaluating the performance of disk diffusion for MRSA detection have shown that such method is less reliable for heterogeneous lineages. Studies using lineages that had been confirmed to be heterogeneous reported 61% sensitivity of a total of 80 *mecA*-positive lineages, many of which were heterogeneous. Another study reported 88.5% sensitivity for class-1 lineages (extremely heterogeneous) and 96.4% for class-2 lineages [35]. Velasco et al. [36] observed that the methods based on agar disk diffusion, E-test and microdilution are frequently not completely reliable for detecting lineages with the *mecA* gene. Of the 51 *mecA*-positive samples, three were reported as false negative by such methods. According to the same authors, the cefoxitin disk (30 µg), recommended by NCCLS [37] as a screening method, showed better results, with 100% sensitivity and 98.0% specificity. Similar results were previously observed by Cauwelier et al. [38], who also reported better results with cefoxitin disks as compared with oxacillin disks. Similar findings were also recently reported by Pereira et al. [39] with *S. aureus* isolated from patients from the pediatrics and neonatal units of the Botucatu School of Medicine, and detection by the cefoxitin disk diffusion method showed 100% sensitivity and 98% specificity as compared with 94.4% and 98.8% sensitivity and specificity, respectively, for the oxacillin disk.

A study conducted by Martins et al. [20] on *S. aureus* isolates from adult patients from the Botucatu School of Medicine University Hospital showed 86.9% sensitivity and 91.1% specificity by the oxacillin disc diffusion test. The cefoxitin disk and screening method presented a similar sensitivity (91.3%) and the same specificity. The E-test method showed the highest sensitivity (97.8%), with the same specificity (91.3%) found by the other methods.

The performance of the MH agar screening test supplemented with 4% of NaCl and 6 µg of oxacillin is also dependent on the heterogeneity levels of the lineages used, lower sensitivity values (≤ 95%) in studies with a larger number of heteroresistant lineages, but with values > 97% were reported [29, 34, 35]. A study performed by Pereira et al. [39] on *S. aureus* isolated from the pediatrics and neonatal units of the Botucatu School of Medicine showed 100% sensitivity and 98.8% specificity by the screening method. For CoNS, the MH agar screening method supplemented with 4% of NaCl and 4 µg of oxacillin, sensitivity and specificity were 96.5% and 83.3%, respectively [16]. The usual use of automated systems for detecting such lineages in clinical microbiology laboratories shows lower sensitivity, specificity and diagnostic efficacy in relation to reference systems [29], requiring result confirmation through the use of methods recommended by CLSI.
Considering that the phenotypic methods for MRSA detection may sometimes provide questionable results, molecular tests have been proposed for detecting the mecA gene or its PBP 2a product. Investigation of the mecA gene by using the Polymerase Chain Reaction (PCR) technique is considered to be gold standard for MRSA detection [37, 40]. According to CLSI [40], the tests for detection of the mecA gene or of the protein codified by such gene (PBP 2a) are the most adequate for determining oxacillin resistance, and they can be used to confirm the results obtained by disk tests in more serious infections.

4. Vancomycin Resistance

With the development of vancomycin-resistant Enterococcus in 1988 and the transfer potential of such resistance to other bacteria, vancomycin resistance surveillance has been an object of great scientific interest worldwide. In-vitro studies have shown that the vanA gene can be transferred from enterococci to a variety of Gram-positive germs [41], including S. aureus [42].

In 1996, the first clinical S. aureus isolate with reduced vancomycin sensitivity, with MIC value in the intermediate range (MIC = 8 μg/ml) and referred to as vancomycin-intermediate S. aureus (VISA), was reported in Japan [43]. Additionally, in June 2002 [44], eight patients with infections caused by S. aureus with reduced vancomycin sensitivity were confirmed in the United States. One month later, the Centers for Disease Control and Prevention (CDC) published the first reported on vancomycin-resistant S. aureus (VRSA, with MIC = or ≥ 32 μg/ml) in a patient in Michigan, United States. The sample isolated from the patient contained the vanA gene as well as the mecA gene for oxacillin resistance. The presence of the vanA gene in this VRSA suggests that resistance may have been acquired through the passage of genetic material from vancomycin-resistant enterococci to S. aureus. In October of the same year [44], the second clinical isolate of VRSA was reported in a patient in Pennsylvania. The VRSA isolate also contained the vanA and the mecA genes. The presence of the vanA gene suggests that the resistance determinant was acquired from vancomycin-resistant Enterococcus isolated from the same patient. April 2004, the third VRSA isolated from a patient in New York was reported. The isolate also contained the oxacillin- and vancomycin-resistance mecA and vanA genes, respectively. According to CDC, the three VRSA isolated did not seem to be epidemiologically related [44, 45]. The CDC [46] has recently confirmed the 11th case of vancomycin resistant Staphylococcus aureus (VRSA) infection since 2002 in the United States (Table 2). This serves as a reminder about the important role of clinical laboratories in the diagnosis of VRSA cases to ensure prompt recognition, isolation, and management by infection control personnel. Appropriate antimicrobial prescribing by healthcare providers, adherence to recommended infection control guidelines, and, ultimately, the control of both MRSA and VRE are necessary to prevent further emergence of VRSA strains.

In Brazil, VISA strains were described by Oliveira et al. [23], of which four strains were isolated from patients at a burn unit and one strain from a patient at an orthopedics unit. All patients were submitted to vancomycin treatment for more than 30 days. Intermediate vancomycin sensitivity development may be related to long contact of the microorganism with this antimicrobial, thus raising epidemiological concern towards the detection and control of such resistance in Brazilian hospitals. No specific genes related to that resistance were described for the VISA strains, and investigation suggests that the resistance mechanism may be related to bacterial cellular wall thickening and the possibility of vancomycin absorption by such modified wall [47].

Similarly to S. aureus, when CoNS are multiresistant to commonly used antibiotics, glycopeptide vancomycin is considered as the antibiotic of choice. However, Schaberg et al. [48] and Veach et al. [49] isolated lineages of vancomycin-resistant S. haemolyticus from patients submitted to prolonged therapy with that antimicrobial. The S. haemolyticus lineages isolated from the patients undergoing vancomycin treatment showed reduced susceptibility to such drug when compared to other lineages isolated prior to antibiotic therapy [48, 49]. Despite being rare, these lineages may be the sign of the beginning of resistance to an important antibiotic used in staphylococcal infection treatment.
Table 2 Historical U.S. VRSA case count and geographical information:

<table>
<thead>
<tr>
<th>Case</th>
<th>State</th>
<th>Year</th>
<th>Age</th>
<th>Source</th>
<th>Diagnosis</th>
<th>Underlying Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MI</td>
<td>2002</td>
<td>40</td>
<td>Plantar ulcers &amp; Catheter tip</td>
<td>Plantar soft tissue infection</td>
<td>Diabetes, dialysis</td>
</tr>
<tr>
<td>2</td>
<td>PA</td>
<td>2002</td>
<td>70</td>
<td>Plantar ulcer</td>
<td>Osteomyelitis</td>
<td>Obesity</td>
</tr>
<tr>
<td>3</td>
<td>NY</td>
<td>2004</td>
<td>63</td>
<td>Urine from a nephrostomy tube</td>
<td>No infection</td>
<td>Multiple sclerosis, Diabetes, kidney stones</td>
</tr>
<tr>
<td>4</td>
<td>MI</td>
<td>2005</td>
<td>78</td>
<td>Toe wound</td>
<td>Gangrene</td>
<td>Diabetes, vascular disease</td>
</tr>
<tr>
<td>5</td>
<td>MI</td>
<td>2005</td>
<td>58</td>
<td>Surgical site wound after panniculectomy</td>
<td>Surgical site infection</td>
<td>Obesity</td>
</tr>
<tr>
<td>6</td>
<td>MI</td>
<td>2005</td>
<td>48</td>
<td>Plantar ulcer</td>
<td>Osteomyelitis</td>
<td>Chronic ulcers</td>
</tr>
<tr>
<td>7</td>
<td>MI</td>
<td>2006</td>
<td>43</td>
<td>Triceps wound</td>
<td>Necrotizing fasciitis</td>
<td>Diabetes, dialysis, chronic ulcers</td>
</tr>
<tr>
<td>8</td>
<td>MI</td>
<td>2007</td>
<td>48</td>
<td>Toe wound</td>
<td>Osteomyelitis</td>
<td>Diabetes, obesity, chronic ulcers</td>
</tr>
<tr>
<td>9</td>
<td>MI</td>
<td>2007</td>
<td>54</td>
<td>Surgical site wound after foot amputation</td>
<td>Osteomyelitis</td>
<td>Diabetes, hepatic encephalopathy</td>
</tr>
<tr>
<td>10</td>
<td>MI</td>
<td>2009</td>
<td>53</td>
<td>Plantar foot wound</td>
<td>Plantar soft tissue infection</td>
<td>Diabetes, obesity, lupus, rheumatoid arthritis</td>
</tr>
<tr>
<td>11</td>
<td>DE</td>
<td>2010</td>
<td>64</td>
<td>Wound drainage</td>
<td>Prosthetic joint infection</td>
<td>Diabetes, end-stage renal disease, dialysis</td>
</tr>
</tbody>
</table>

Source: CDC [46]

In 2006, a vancomycin-resistant *S. cohnii* strain was isolated from the pleural fluid of a 5-year-old patient at San Jerónimo de Monteria Hospital in Colombia. That patient had been in prolonged therapy with vancomycin, and MIC, by the E-test, was of 64 μg/ml. The same isolate showed resistance to oxacillin, teicoplanin, ceftazidime and trimethoprim-sulfamethoxazole [50].

Another report, this time on CoNS samples from healthy patients, was made by Palazzo et al. [51] in Brazil. A total of four vancomycin-resistant *Staphylococcus* spp. samples (1 *S. epidermidis*, 1 *S. haemolyticus* and 2 *S. capitis*) were isolated from workers at a private school and a hospital located in the region of Ribeirão Preto, SP, Brazil. The minimum inhibitory concentration presented by these samples, when tested by the E-test technique, ranged from 16 μg/mL to ≥256 μg/mL.

The choice of an adequate laboratory method influences the capacity of surveillance on glycopeptide resistance and makes the effective worldwide prevalence of isolates still unknown [19]. Although disk-diffusion testing is still the most frequently used in laboratory routine, it is not the recommended test for VISA or VRSA detection [40]. The methods recommended by CLSI [40] are MIC determination or the BHI agar screening test containing 6 μg/mL of vancomycin. All *Staphylococcus* spp. showing growth during screening must be confirmed in relation to the presence of pure culture and tested by a method for vancomycin MIC determination. Hence, similarly to MRSA, VISA expresses resistance in a heterogeneous fashion. The colonies expressing vancomycin resistance grow more slowly, thus hindering detection by routine methods used for sensitivity tests in most clinical laboratories (disk diffusion). For this reason, *Staphylococcus aureus* with MIC between 2 and 4 μg/mL must be carefully analyzed [19]. *Staphylococcus* spp. with reduced vancomycin sensitivity presents limited options and requires precaution for infection control in order to reduce transmission and minimize the installation of possible outbreaks.
Other recent more classes of antibiotics, such as streptogramins (quinupristin/dalfopristin), oxazolidone (linezolid), glycolcycline (tigecycline) and lipopeptides (daptomycin), can be options in VISA or VRSA treatment. However, staphylococci have already selected some specific forms to resist to new antibiotics. Although these resistant isolates are rare, under a pessimistic viewpoint, it can be said that once resistant strains already exist, the useful life of these new antibiotics is already threatened. Under a more optimistic viewpoint, it can be stated that the availability of various antimicrobial alternatives against multiresistant microorganisms can delay resistance dissemination [52]. The careful use of these new classes is, nevertheless, imperative so that therapeutic options can be preserved.

5. Conclusion

Oxacillin resistance in Staphylococcus spp. is generally accompanied by resistance to multiple antimicrobial agents, and this fact results in the increased use of glycopeptides for empirical therapy and even prophylaxis. However, glycopeptide resistance selection and the potential transmission of that resistance between species have shown the need to restrict the use of such antimicrobials. These data point out the need to perform antimicrobial susceptibility tests and identify methicillin-resistant staphylococci as early as possible so that therapeutic options can be preserved.

Acknowledgements The support by FAPESP is gratefully acknowledged.

References


