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Epidemiological Aspects of Oxacillin-Resistant *Staphylococcus* spp.: The Use of Molecular Tools with Emphasis on MLST

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1. Introduction

Studying infections caused by the *Staphylococcus* spp. genus is highly important for human health given that such organisms are causal agents of superficial infections, such as abscesses and impetigo, as well as of systemic infections, namely bacteremia and osteomyelitis. This genus is divided into two large groups. The first group is characterized by the production of enzyme coagulase, and its main representative is *S. aureus*, which is frequently associated with a large variety of infections. The second group, known as coagulase-negative staphylococci (CoNS), is usually associated with immunocompromised patients or those who use catheters. (Kloos & Bannerman, 1995). The main CoNS species associated with infection in humans are *S. epidermidis, S. haemolyticus, S. saprophyticus, S. cohnii, S. xylosus, S. capitis, S. warneri, S. hominis, S. simulans, S. saccharolyticus, S. auricularis, S. caprae, S. lugdunensis* and *S. schleiferi* (Kloos & Bannerman, 1995).

Several studies have reported increased prevalence of CoNS infection in hospitals, which is usually associated with resistance to the antibiotic of choice for treatment. Hence, this is a serious clinical and epidemiological problem (Jain et. al., 2008).

The use of methicillin and other semi-synthetic penicillins, such as oxacillin and penicillinase-resistant methicillin, which began in 1959, represented a significant phase in anti-staphylococcal therapy. The first report on methicillin resistance was in 1961, a short time after its use was implemented (Hiramatsu et al, 2001). In Brazil, it is estimated that the frequency of oxacillin resistance is high among *S. aureus* samples, particularly in large and in university hospitals. Gales (2009) described an oxacillin resistance rate of 31% in a multi-center study involving four Brazilian hospitals. As regards CoNS, 78.7% of the samples were resistant. At the Botucatu School of Medicine University Hospital – SP, approximately 45% of the *S. aureus* samples from hemocultures were positive for the *mecA* gene (Martins et al., 2010). In a study conducted at the neonatal intensive care unit of the same hospital, a total of 18% of MRSA samples was found (Pereira et al., 2009). According to a study by Sader et al. (2004) in Latin America and Brazil, respectively, 80.4% and 84.6% of the CoNS samples from hemocultures were oxacillin resistant.

Oxacillin resistance in CoNS samples varies significantly among the different species in the genus, a fact that reinforces the importance of their correct identification (Secchi et al., 2008).

Although *S. epidermidis* is the most frequently found species (Vuong & Otto, 2002), others are also associated with human infection, such as *S. haemolyticus*, which can be multi-resistant and present intermediate resistance to vancomycin (Secchi et al., 2008). The main resistance mechanism is the presence of the *mecA* gene, inserted in the chromosomal cassette, known as staphylococcal chromosomal cassette mec (SCC*mec*). The detection of this gene and the typing of the chromosomal cassette by means of various molecular methods are important tools for the diagnosis and epidemiology of oxacillin-resistant *Staphylococcus* spp.

There are eleven SCC*mec* types, with subtypes, which are characterized by molecular tools, such as Multilocus Sequence Typing (MLST), pulsed-field gel electrophoresis (PFGE), *spa* typing and Multiplex PCR for SCC*mec* detection. They are useful in the characterization and detection of alterations in molecular structure. By using these methods, it was possible to identify pandemic clones as well as to characterize strains causing outbreaks in hospitals. Among these methods, the MLST is noteworthy due to its high reproducibility and capacity of detecting pandemic clones. Given the fact that vancomycin is a therapeutic option for oxacillin-resistant samples, with the emergence of vancomycin-resistant *Staphylococcus* spp., the characterization of circulating strains and clones is highly important. This chapter aimed at addressing aspects related to the molecular epidemiology in *Staphylococcus* spp. since these microorganisms have been increasingly frequent as agents of nosocomial and community infection.

2. Epidemiological aspects

Oxacillin resistance in CoNS is a problem in hospitals around the world, and there are reports of oxacillin-resistant samples in all continents (Witte, 1999). The use of methicillin, a semi-synthetic penicillin, commenced in 1959, and only two years after its first use, the first report of a methicillin-resistant *Staphylococcus* spp. sample was published (Hiramatsu et al., 2001).

Oxacillin resistance rates vary among various studies, but they are usually high, above 50%. Chaudhury & Kumar (2007) reported that, in a study conducted in a tertiary Indian hospital, 64.6% of the CoNS samples were oxacillin resistant. The most prevalent species was *S. haemolyticus*, isolated from urine samples. Another study also performed in India, described resistance levels of approximately 63% (Jain et al., 2008).

In the European continent, several reports described high oxacillin resistance levels in hospital wards. In a study analyzing samples collected from various hospitals in Eastern Europe in 2005, Sader et al. (2007) reported oxacillin resistance levels in CoNS which varied from 54.8% in Sweden to 83.3% in Greece. A study conducted at a university hospital in Turkey found 54.4% of resistant samples from a total of 158 isolated samples (Ercis et al., 2008). The rates found by a multi-center study conducted in the USA in 2007 and 2008 were of 74% of CoNS oxacillin-resistant samples (Sader & Jones, 2009).

Nevertheless, in the last few years, resistance levels have stabilized. In Spain, a multi-center study on 146 hospitals detected oxacillin resistance in 61.3% of the samples in 2002 and 66.7% in 2006 (Cuevas et al., 2008). In Brazil, recent studies showed resistance rates of 69% (Caierão et al., 2004), 78.4% (Perez & d'Azevedo 2007) and even 88.1% (Antunes et al., 2007). In the case of non-*epidermidis* CNS, Secchi et al. (2008) reported 71% of resistant samples.

Based on the described reports, it is clear that oxacillin resistance is prevalent in hospital in all continents, a fact that reinforces the importance of good antibiotic therapy practices and of infection control measures in hospitals.

3. Oxacillin resistance

Oxacillin resistance is associated with the drug's reduced capacity to adhere to the penicillin-binding protein (PBP), thus also losing its capacity to lyse the bacterial cell. There are three mechanisms of resistance to semi-synthetic penicillinases, a group of drugs in which oxacillin is included. The first is related to the hyperproduction of β-lactamases, enzymes that cleave the drug's β-lactam ring, thus inactivating it (McDougal & Thornsberry, 1986). The second mechanism, known as MOD-SA, occurs when normal PBPs have reduced affinity with oxacillin (Tomasz et al., 1989). The third and most important mechanism is the presence of the *mecA* gene. This gene codifies a changed PBP, known as PBP 2a, thus preventing its binding to oxacillin (Zhang et al., 2009).

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 (Tomacz et al., 1991). 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. 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^{-5}) than in class-1 lineages (Tomacz et al. , 1991). 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 (Tomacz et al., 1991).

The phenotypic expression codified by the *mecA* gene is affected by various factors, including pH, temperature and osmolarity (Swenson, 2002). 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 (Swenson, 2002).

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.

The *mecA* gene is inserted in a transposable genetic element known as Staphylococcal Chromosomal Cassette *mec* (*SCCmec*). This element varies in its constitution and is divided into eleven types. The typing of *SCCmec* types is useful as an epidemiological tool (Mombach Pinheiro Machado et al., 2007) given that the different types are more prevalent in hospital and community environments. The SCC*mec* types (IWG-SCC, 2009) differ from one another in relation to the number of genes that they carry in their gene architecture (Hiramatsu et al., 2001). Some of these types are carriers of resistance genes that are determinant for multiple antibacterial drugs. In addition to beta-lactam antibiotics, macrolides, lincosamides, streptogramins, aminoglycosides and tetracycline are noteworthy. Hence, when a bacterial cell acquires such SCC*mec*, it at once acquires a multiple-resistance phenotype (Ito et al., 2003).

The SCC*mec* types have been defined by the combination of two parts: the *ccr* complex and the *mec* complex, with three phylogenetically distinct *ccr* genes classified as: *ccrA, ccrB* and *ccrC*. Additionally, there are five classes of *mec* gene complexes (classes *A*, *B*, *C1*, *C2* and class *D*) (Ito et al., 2004; IWG-SCC, 2009). The different SCC*mec* types are classified as: type-I SCC*mec* (class *B* and *ccrA1B1 mec* gene complex), type-II SCC*mec* (class *A* and *ccrA2B2 mec* gene complex), type-III SCC*mec* (class *A* and *ccrA3B3 mec* gene complex), type-IV SCC*mec* (class *B* and *ccrA2B2 mec* gene complex), type-V SCC*mec* (class *C2* and *ccrC mec* gene complex), type-VI SCC*mec* (class *B* and *ccrA4B4 mec* gene complex), type-VII SCC*mec* (class *C1* and *ccrC mec* gene complex) and type-VIII SCC*mec* (class *A* and *ccrA4B4 mec* gene complex). The remaining region of SCC*mec* is called the J region (Joining region), which constitutes non-essential components of the cassette that can carry additional antimicrobial resistance determinants (Hanssen & Sollid, 2006; IWG-SCC, 2009). Recently, types IX (class C2, ccr1 *mec* gene complex), X (class C1, ccr7 *mec* gene complex) and XI (class E, ccr8 *mec* gene complex) have been described (IWG-SCC, 2011) (Table 1).

SCC*mec* type	*mec* gene complex	*ccr* gene complex
I	B	1 (A1B1)
II	A	2 (A2B2)
III	A	3 (A3B3)
IV	B	2 (A2B2)
V	C2	5 (C1)
VI	B	4 (A4B4)
VII	C1	5 (C1)
VIII	A	4 (A4B4)
IX	C2	1 (A1B1)
X	C1	7 (A1B6)
XI	E	8 (A1B3)

Source: http://www.sccmec.org/Pages/SCC_TypesEN.html. Accessed on 06/20/2011

Table 1. SCC*mec* types identified in *S.aureus*

The first three types were detailed by Ito et al. in 2001, and the same author also reported and described type V in 2004. Ma et al. (2002) described type IV, which is mainly found in

community samples. Types I, II and III are mainly responsible for nosocomial infections, and they are significantly larger than the last types IV and V. Type-I cassette was described with a size of 34.364 bp, and it is the largest of the three. This cassette does not feature any inserted transposons or plasmids that provide resistance to other drugs besides methicillin or to heavy metals. It has a sub-type known as IA, which differs from type I for having an integrated plasmid, pUB110 (Shore et al., 2005).

The second cassette, referred to as II, has 53.017 bp, and in addition to the *mecA* and *mecRI* genes, which cause methicillin resistance, it contains transposon Tn 554. The latter is responsible for the resistance of this sample type to erythromycin and streptomycin. This cassette has a sub-type that is referred to as IIA, with a size of 40 Kb, thus being a little smaller than type II (Shore et al., 2005).

Type-III cassette, the largest of the five types, has a size of 66.896 bp and contains genes *mecA*, *mecRI*, transposons Tn 554 and ψTn 554 and plasmid pt 181. Transposon ψTn 554 induces cadmium resistance, and plasmid pt 181 is responsible for tetracycline and mercury resistance. In addition to the information described above, the authors also mentioned differences between the *ccr* gene types, by describing, in type-III *SCCmec,* a gene that is not present in the other types and is referred to as *ψccr*. They also suggested that some of the resistance characteristics in type III can be used as selective markers. This cassette type presents two sub-types: IIIA, which does not have plasmid pt 181 and flanks with element IS 431, and IIIB, where there are no copies of plasmids pt 181 or tn 554 (Shore et al., 2005).

Type-IV SCC*mec* is mainly responsible for community infections. It is a small element that does not carry other resistance genes, except for *mecA*. It is also divided into multiple sub-types, which suggests that type-IV SCC*mec* is highly transmittable (Ito et al., 2004). The four sub-types of type-IV, -IVA, -IVB, -IVC and -IVD cassettes differ for presenting different sequences on the left extremity of the *ccr* complex, which is known as L – C Region (Shore et al., 2005).

Type-V SCC*mec* was identified by Ito et al. (2004) in an Australian isolate. It has the size of 27.624 bp, and it is a little larger than SCC*mec* IV although smaller than the other existing types. Similarly to type IV, it has only genes that codify methicillin resistance; however, differently from the other elements in this family, type V has a new *ccr* gene type that is characterized as type c and is individually found, contrarily to other elements, which have a pair of such genes. Another new element found only in type V is a restriction and modification system codified by genes V22 and V23. Recent studies associate the presence of this *mec* cassette type with community infections (O'Brien et al., 2005; Ho et al., 2007).

Oliveira et al. (2006) characterized type-VI SCC*mec*, which is found in samples belonging to the pediatric clone of MRSA (Methicillin Resistant *S.aureus*) samples and previously typed as SCC*mec* IV. The authors described differences in the *ccrAB* complex, identified as type 4, and the presence of the type-B *mec* complex, which does not have the *mecl* gene.

Type-VII SCC*mec* was identified by Higuchi et al. (2008) through a detailed analysis of community *S.aureus* samples isolated in Taiwan. It has a size of 41.347 bp, and its *mec* complex is homologous to that found in type V, but presents substitutions, insertions and rearrangements that differentiate it. The main characteristics of this cassette type are the presence of the *ccrC* complex, of transposon ΨIS431 and of a unique sequence on the right side of the cassette which is referred to as *orf*35.

Recently, Zhang et al. (2009) have described a new SCC*mec* type which has been denominated as SCC*mec* VIII and was found in an MRSA sample from a hospital. This new cassette has a size of 32.168 base pairs, and genes *mecA mecR1 mecI* of the *mec* complex and *ccrA4 ccrB4* of the *ccr* complex are present in addition to transposon Tn554, which is also present in type II.

4. Molecular epidemiology

In addition to the characterization of SCC*mec* types as an epidemiological tool, other methods are available for molecular epidemiology studies, such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST) and *spa* typing, which allow for the identification of circulating bacterial clones in hospitals and in the community.

PFGE is a method that uses restriction enzymes to cleave the extracted DNA, and in the case of the *Staphylococcus* genus, the enzyme used is *Sma*I. The fragments are separated in agarose gel. The direction of the electrical field is periodically changed, forming a 120º angle. This allows for fragments of up to 12mb to be separated, contrarily to conventional electrophoresis, which is not capable of separating fragments that are larger than 50kb (Herschleb et al., 2007). This method is generally used in local studies, such as in hospital outbreaks, which allows for identifying the circulating type.

The sequencing of constitutive bacterial genes can also be used as an important epidemiological tool. The method known as *spa* typing investigates polymorphisms on a single locus. It can discriminate between PFGE and MLST and is also applied in local studies and for detecting pandemic clones (Malachowa et al., 2005).

Another methodology that uses the sequencing and analysis of genetic polymorphisms is MLST. It analyzes the sequence of seven constitutive genes (*gmk, pta, dfp, gtr, mutS, pyrR,* and *xpt*) and compares them with the sequences of each allele in the locus, which are previously numbered. The combination of the alleles identified in each gene determines the profile of the sample. (Aanensen & Spratt, 2005). It is the most adequate methodology for detecting pandemic clones given that the investigated genes are constitutive, with low mutation rates when compared to the analysis of the whole chromosome, in the case of PFGE (Miragaia et al., 2007).

5. Pandemic clones of MRSA samples

Currently, *Staphylococcus spp*. samples have been typed in numerous epidemiological studies by means of the methods described above. By using these tools, 05 large pandemic clones have been described. These are the Brazilian clone, characterized by *SCCmec* IIIA, MLST 2-3-1-4-4-3 (ST 239); the Iberian clone with type-IA *SCCmec*, and MLST 3-3-1-1/12-4-4-16 (ST 247); the clone known as New York / Japan, with type-III SCC*mec*, pattern MLST 1-4-1-4-12-1-10 (ST 05); the Hungarian clone, with SCC*mec III*, MLST 2-3-1-1-4-4-3 (ST 239); the pediatric clone, with type-IV SCC*mec*, MLST 1-4-1-4-12-1-10 (ST 05) and the last large clone known as EMRSA-16, *SCCmec* II MLST 15-12-16-02-16-02-25-17-24-24-24 (ST 36) (Oliveira et al., 2002; Melter et al., 2003; Velásquez-Meza et al. 2004; Aires de Souza & De Lencastre, 2004, Aires Ribeiro et al., 2005).

In our scenario, the predominant MRSA samples belong to the Brazilian clone (Oliveira et al., 2001). The first report on the emergence of this clone dates to 1992-93 in various hospitals

in Brazil. The samples were characterized as belonging to the same clone by methods such as PFGE and by showing patterns of transposon *Tn554* and the polymorphism of the *mecA* gene (Teixeira et al., 1995). In other countries, the Brazilian clone is also disseminated, as is the case of the Czech Republic, where the isolation of this clone represented 80% of the MRSA samples found in 1996-1997 (Melter et al., 2003). The Brazilian clone was also described in India, in two hospitals in the region of Bengalore, conjointly with the Hungarian clone (*SCCmec III*) (Arakere et al., 2005)

Other clones are distributed in several parts of the world. The Iberian clone was firstly described in samples from hospitals in Barcelona and Madrid, Spain, and in Lisbon, Portugal. These samples were typed by the PFGE method and probe hybridization, producing a pattern that characterized them as belonging to the same clone. This clone is also described in other countries, such as the Czech Republic (Melter et al., 2003). The clone known as New York – Japan, firstly isolated in the USA in 1994-98 and in Japan in 1997-98 (Oliveira et al., 2002), was also predominant in Mexico during a study on 98 MRSA samples, thus replacing the local clone, known as Mexican (PFGE M, type-IV SCC*mec*), in nosocomial infections (Velasquez-Meza et al., 2004).

The Hungarian clone, firstly identified in Hungary in 1993-94, was characterized by the same methods used for the clones described above in studies on MRSA samples from hospitals in 06 provinces in that country (De Lencastre, 1997). The pediatric clone was isolated in large numbers in 1996-98 in Colombia, and it is also found in Argentina and Poland in 1994-98 and 1990-98 (Oliveira et al., 2002).

The last of the large pandemic clones, referred to as EMRSA-16, is prevalent in hospitals in the United Kingdom, Mexico and Greece, in addition to being responsible for an outbreak in Sweden from 1997 to 2000 (Aires de Souza & De Lencastre, 2004).

6. Epidemiology and MLST

The MLST method was developed by Maiden et al. (1998) by the sequencing and analysis of the loci of eleven constitutive genes of *Neisseria meningitidis*, and it is presently applied in molecular epidemiology studies on various pathogens, among which are *S. aureus* and *S. epidermidis*.

This methodology has been used in numerous studies on molecular epidemiology with good results due its good reproducibility, thus allowing for the detection of pandemic clones from circulating *S. aureus* and *S. epidermidis* samples.

Geng et al. (2010) reported the presence of clone ST59-MRSA-IV in China in community samples isolated from 47 children with impetigo and abscesses. In Malaysia, a study on 36 samples isolated from a hospital in Klang Valley for a five-month period reported a rate of 83.3% of samples belonging to clone ST 239 *SCCmec* III (Neela et al., 2010).

MLST has been used for studies in which circulating clones are identified in replacement of another already established clone. Sola et al. (2006) reported the emergence of a new clone identified as ST5 in hospitals in Cordoba, Argentina, in replacement of the Brazilian clone (ST239), which circulates in that region. In that study, 103 MRSA samples isolated from April to June 2001 and 31 MSSA samples isolated from 1999 to 2002 were used.

Previous studies have used MLST for comparison between samples and the prevalence between different pandemic clones. Campanille et al. (2009) analyzed 301 MRSA samples isolated from 19 Italian hospitals from 1990 to 2001. An increase of clone ST228, known as Italian clone, was observed from 2000 to 2007, conjointly with the decrease of clone ST247 (Iberian clone) when compared to the 1990-1999 period.

Clone ST228 is also associated with patients with cystic fibrosis. A study conducted on 93 MRSA samples isolated from patients with that disease at a treatment center in Madrid, Spain, identified 15 different PFGE patterns. A sample of each of these patterns was typed by MLST, with clone ST228 showing higher prevalence, with eight pulsetypes, followed by ST5, with two pulsetypes, and ST247, ST72 and ST255 with one pulsetype each (Molina et al., 2008).

In addition to epidemiological studies in hospitals and the detection of these pandemic clones from MRSA samples, the MLST method has been used to detect the transmission of oxacillin-resistant samples from animal reservoirs to humans, including its detection in hospitals. Some studied have detected the presence of MRSA clones in swines, thus emphasizing the importance of such animals as reservoirs. Smith et al. (2009) reported the presence of ST398 samples in swines and farmers in the mid-western region of the United States, which suggests transmission between animals and their breeders. In Germany, 1,600 swabs from swines from 40 farms were analyzed in a study where samples typed as ST398 were also identified (Köck et al., 2009).

MLST is usually used for detecting pandemic clones with good results. But this method can also be utilized for characterizing samples involved in hospital outbreaks.

Peacock et al. (2002) compared the MLST and PFGE methods in a study conducted on 104 *S. aureus* samples isolated from nasal swabs from patients under renal therapy. The isolated samples were typed by the MLST and PFGE methods with a similar discriminatory power between the two techniques for identification of circulating samples in hospitals.

Vindel et al. (2009) analyzed 463 *S.aureus* samples isolated from 145 Spanish hospitals in 2006. In addition to MLST, several methodologies such as PFGE, spa typing, *SCCmec* characterization and *agr* typing were used. MLST showed good correlation with the methodologies used for detecting circulating samples, with applicability in localized nosocomial studies.

The combination of the two molecular epidemiology techniques can increase discriminatory power for analysis of different clones. Cookson et al. (2007) reported that the combination of the MLST and *SCCmec* methods are more appropriate for multi-center studies. The authors analyzed MRSA samples from various European countries by means of PFGE, MLST, *SCCmec* analysis and *spa* typing.

7. Vancomycin-resistant *S. aureus* (VRSA)

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. Acquired microbial resistance to vancomycin is a growing problem, in particular, within healthcare facilities such as hospitals. The widespread use of vancomycin makes resistance to the drug a significant worry, especially

for individual patients if resistant infections are not quickly identified and the patient continues the ineffective treatment. Vancomycin-resistant Enterococcus (VRE) emerged in 1987 and the transfer potential of such resistance to other bacteria, vancomycin resistance surveillance has been an object of great scientific interest worldwide. Vancomycin resistance emerged in more common pathogenic organisms during the 1990s and 2000s, including vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA).

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 VISA was reported in Japan (Hiramatsu et al. 1997). 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 (CDC 2002; CDC 2004). The CDC (2010) 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

Case	State	Year	Age	Source
1	Michigan	2002	40	Plantar ulcers & Catheter tip
2	Pennsylvania	2002	70	Plantar ulcer
3	New York	2004	63	Urine from a nephrostomy tube
4	Michigan	2005	78	Toe wound
5	Michigan	2005	58	Surgical site wound after panniculectomy
6	Michigan	2005	48	Plantar ulcer
7	Michigan	2006	43	Triceps wound
8	Michigan	2007	48	Toe wound
9	Michigan	2007	54	Surgical site wound after foot amputation
10	Michigan	2009	53	Plantar foot wound
11	Delaware	2010	64	Wound drainage

Source: CDC (2010)

Table 2. Vancomycin resistant *Staphylococcus aureus* (VRSA) infection in the United States

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.

8. Conclusions and perspectives

Given the high rates of oxacillin resistance reported in various countries and the emergence of vancomycin-resistant MRSA samples, further detailed studies on the characteristics of circulating strains in hospitals and the characterization of clones prevailing in larger regions are necessary since the different MRSA clones vary in virulence and antimicrobial resistance. The molecular epidemiology methods are useful tools in these types of study, and the MLST technique is especially useful due to its versatility, easy performance and high reproducibility, with applications in localized studies, such as in the characterization of outbreaks and in the detection of circulating clones in large regions.

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This book represents an overview on the diverse threads of epidemiological research, brings together the expertise and enthusiasm of an international panel of leading researchers to provide a state-of-the art overview of the field. Topics include the epidemiology of dermatomycoses and Candida spp. infections, the epidemiology molecular of methicillin-resistant Staphylococcus aureus (MRSA) isolated from humans and animals, the epidemiology of varied manifestations neuro-psychiatric, virology and epidemiology, epidemiology of wildlife tuberculosis, epidemiologic approaches to the study of microbial quality of milk and milk products, Cox proportional hazards model, epidemiology of lymphoid malignancy, epidemiology of primary immunodeficiency diseases and genetic epidemiology family-based. Written by experts from around the globe, this book is reading for clinicians, researchers and students, who intend to address these issues.

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