Staphylococcus spp.: an update on the molecular epidemiology and mechanisms of antimicrobial resistance

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Staphylococcus aureus and Coagulase-Negative Staphylococci (CoNS) represent harmful pathogenic agents responsible for human and animal infections. The Community-Acquired Methicillin Resistant *S. aureus* (CA-MRSA) is now considered a worldwide health issue. Methicillin resistance is attributed to the acquisition of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), a resistance island that contains the structural *mecA* gene. Recent studies have found a homologue gene to *mecA* denominated MECC, which is located in a cassette chromosome *mec*, designated SCC*mec* type XI. Some new treatment alternatives for methicillin and for vancomycin, a high toxic glycopeptide drug to which some resistant strains, include the lipoglycopeptides televancin and dalbavancin, the lipopeptides daptomycin and tripopeptin C, the oxazolidones linezolid and torezolid, the fluoroquinolones delafloxacin and moxifloxacin, the estreptogramines quinupristin/dalfopristin, the new cephalosporine ceftaroline, the glycylcycline tigecyclin and the DHFR inhibitor iclaprim. Despite their recent introduction, reduced susceptibility to these drugs has been identified in staphylococcal strains. The high spread of resistant strains among individuals lacking classic risk factors for these infections emphasizes the importance of epidemiological surveillance of *S. aureus* and CoNS, and the molecular characterization of resistant strains. The distinction between hospital and community isolates is becoming unclear, which raises the need for the investigation of the dynamics and epidemiology of resistance among these agents.

Keywords: *Staphylococcus* spp.; antimicrobial resistance; methicillin; *mecA*; MECC; vancomycin; novel antimicrobials; biofilm; molecular epidemiology

1. Introduction

Staphylococci are Gram-positive bacteria that cause suppurative infections in humans and other animals. These immobile, facultative anaerobic bacteria are characterized by their ability to grow in media containing up to 10% of salt and to produce catalase, a feature used for their laboratory identification. The most virulent species, *Staphylococcus aureus*, produces a yellow carotenoid pigment and is the cause of chronic and acute infections, such as boils, deep tissue abscesses, enterocolitis, bacteriuria, osteomyelitis, pneumonia, meningitis, septicemia, and arthritis. Species of the group of coagulase-negative staphylococci (CoNS), whose main member is *S. epidermidis*, are opportunistic pathogens. Although previously considered avirulent and noninvasive organisms, their importance as causative agents of serious infections has increased [1;2].

The success of staphylococci as pathogens is mainly due to their versatility. As part of their adaptation to the era of antibiotic therapy, staphylococci were able to evolve and acquire resistance to almost all antimicrobials used for their treatment. The term "superbug" was created to classify the enormous potential of staphylococci to resist antibiotic therapy. Before the discovery of penicillin in the 1940s, staphylococcal infections often resulted in the death of the patient. The sudden emergence and dissemination of penicillin-resistant strains has led to the development of other antibiotics, including streptomycin, chloramphenicol and tetracyclines, which resolved the resistance problem temporarily. However, the emergence of strains resistant to these drugs within a few years has made the treatment of these infections difficult [3].

The introduction in the 1960s of new beta-lactam antibiotics that were resistant to penicillinases, such as methicillin and oxacillin, appeared to be the solution for multidrug-resistant staphylococci since less than 1% of the isolates were resistant, a fact that generally did not affect the efficiency of these drugs to treat infections [4]. However, the successful spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in the 1970s had led to dramatic increases in the number of MRSA infections in subsequent decades [5].

First, methicillin resistance was only an issue among hospital-acquired infections. Today, MRSA infections are known to occur in the community at increasing proportions [6]. Many of the strains causing community-acquired infections are susceptible to some non-beta-lactam antibiotics, while those causing healthcare-associated infections are often resistant to multiple agents and are difficult to treat [7]. Among the few therapeutic options for multidrug-resistant staphylococcal infections, the glycopeptides vancomycin (since the 1950s) and teicoplanin can only be administered intravenously and require monitoring due to their side effects. Other more recent options with good efficacy approved by the U.S. Food and Drug Administration (FDA) include linezolid (approved in 1999) and daptomycin (approved in 2003), although they also have side effects.

Only five new antimicrobial agents had been approved between 2003 and 2007, compared to 16 over the period from 1983 to 1987 [8]. In 2012, the FDA launched an international program to promote the development and approval of new antimicrobial drugs. Within three months, from May to August 2014, the FDA approved dalbavancin, tedizolid and

oritavancin for the treatment of acute bacterial skin and skin structure infections (ABSSSI), including those caused by *S. aureus*. It is expected that these measures will offer more treatment options for multidrug-resistant staphylococcal infections, which are a major global public health problem [9;10].

In recent years, many pharmaceutical companies have discontinued research programs for antimicrobial drugs, although bacterial dissemination in hospitals and in the community is a matter of great concern. Despite the increasing need for new antimicrobial drugs, spending on research and development by the largest pharmaceutical companies is constantly decreasing [11]. Consequently, the current arsenal of relevant antibiotics contains compounds with serious resistance issues, including resistance to almost all classes of drugs such as beta-lactams, quinolones, macrolides, glycopeptides, and tetracyclines.

2. Resistance to oxacillin/methicillin

Bacterial resistance is mediated by two main mechanisms: mutation in a chromosome locus and horizontal gene transfer, i.e., the acquisition of resistance genes previously present in other microorganisms [12]. Resistance genes located on plasmids usually encode enzymes that inactivate the antibiotic or reduce cell permeability. On the other hand, resistance conferred by chromosomal mutations involves modification of the target.

Methicillin resistance in staphylococci is attributed to the acquisition of the staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile element that carries the *mec*A gene and that can easily be transferred among staphylococcal species. Expression of the *mec*A gene results in the production of a modified penicillin-binding protein, PBP2a, a high molecular weight protein that has low affinity for beta-lactams [13]. The SCC*mec* elements are highly diverse in their structural organization and genetic content and are classified into types and subtypes. The SCC*mec* contains genetic components of the *mec* complex and the gene responsible for recombination of the cassette chromosome (*ccr*). Variations in these gene complexes form the primary basis for the classification of the different types of SCC*mec* [14], an element that is important for the study of the molecular epidemiology of *S. aureus* and, to a lesser extent, of CoNS.
Another important resistance mechanism is the production of beta-lactamase. The production of this enzyme by a microorganism explains its survival in an infection environment despite the use of a beta-lactam antibiotic [15].

2.1 Emergence of a novel resistance gene: MECC

In 2007, an epidemiological study conducted in England, which analyzed isolates from bovine mastitis, led to the isolation of *S. aureus* LGA251. This strain is phenotypically resistant to oxacillin and cefoxitin and is considered the first report of MRSA in a dairy herd in the United Kingdom [16]. However, molecular testing for the *mec*A gene was negative. Genome sequencing of strain LGA251 revealed the presence of a gene homologous to *mec*A, initially called $mecA_{LGA251}$ and later MECC [17]. This gene exhibited 69% similarity to the conventional *mec*A gene at the DNA level, and the encoded PBP2a showed 63% identity at the amino acid level. This result explains the phenotypic resistance of the strain, although molecular techniques were unable to detect the *mec*A gene.

A novel type of cassette chromosome, called type XI, was described after the analysis of 12,691 human isolates collected in Germany between January 2006 and June 2011. This strain was named MRSA CC130 and carries the MECC gene [18]. The origin of MECC MRSA and SCC*mec* type XI are still unclear, but the MECC gene has also been detected in *Staphylococcus stepanovicii*, *Staphylococcus xylosus* and *Staphylococcus sciuri*, as well as in a variety of *Staphylococcus* isolated from different animal species [16;19] in many European countries such as Ireland, France, Sweden, The Netherlands, Germany, Austria, Switzerland, Finland, Spain, Norway, and Belgium.

Further investigations, including whole-genome sequencing of MECC-positive staphylococci, could offer information regarding the origin and evolution of this resistance determinant. These data also show that clinical microbiology laboratories should be aware of the possible occurrence of this type of resistance. Although MECC MRSA have been recognized only recently, they could have been causing human infections for more than 35 years.

3. Frequency of drug resistance

Surveillance studies are important tools for the understanding of antimicrobial susceptibility of strains at regional and global levels. In Latin America, studies have shown methicillin resistance rates of about 48% in *S. aureus* and of 84% in CoNS [20] National and regional data reveal a percentage of MRSA of up to 80% in Africa, 90% in the Americas, 53 to 60% in the Mediterranean region and Europe, respectively, 26% in southeastern Asia, and 84% in the Western Pacific [7].

A study conducted in the United States established the frequency of erythromycin resistance at 65% [21]. In Latin America, this prevalence was 47% among *S. aureus* and 70% among CoNS [20]. In a recent study investigating the susceptibility of *S. aureus* to several drugs in Germany, resistance rates of 96% to fluoroquinolones, 78% to erythromycin, 70% to clindamycin, 4% to gentamicin, and 2% to rifampin have been reported [18]. Another study

evaluating the susceptibility of more than 4,000 *S. aureus* strains demonstrated susceptibility to clindamycin in 81% of the isolates and to levofloxacin in 59% [22].

4. Novel antimicrobial drugs and resistance

Surveillance studies evaluating the susceptibility of a large number of strains to recently approved antimicrobial agents have been published. A recent study conducted in Germany involving a large number of MRSA strains identified resistance to daptomycin in 0.4% of the strains and to linezolid in 0.1% [18]. Another publication from China studying 1,725 MRSA found six isolates that were non-susceptible to vancomycin, one to teicoplanin, one to tigecycline, and one to daptomycin [23]. On the other hand, a global surveillance study demonstrated total susceptibility to linezolid, tigecycline, and vancomycin [24].

4.1 Vancomycin

Although not a recent drug, vancomycin is still classified as a first-line drug for the treatment of MRSA and multidrug-resistant CoNS infections. Strains exhibiting intermediate resistance or heteroresistance to vancomycin have been identified [25; 26]. Although the rates of vancomycin-intermediate *S. aureus* (VISA) are increasing, fully vancomycin-resistant *S. aureus* (VRSA) isolates are still rare [27]. However, only few antibiotics, including linezolid, daptomycin, tigecycline and quinupristin/dalfopristin, are active against vancomycin-resistant strains [28].

Vancomycin is the main option for the treatment of serious infections caused by *S. aureus* [29]. Although the *in vitro* activity of vancomycin is consistent and potent, several studies have shown treatment failure and increased mortality in infections caused by organisms with higher MICs (≥1.5 μg/mL) [30].

Cell-wall thickening has been indicated as the main mechanism mediating the reduced susceptibility and resistance to vancomycin. A study on *S. aureus* Mu50, which exhibits intermediate resistance to vancomycin, revealed the production of an increased amount of peptidoglycan, which adds more murein monomers and layers to the cell wall. As a result, vancomycin molecules are trapped in the peptidoglycan layers, preventing them from reaching the cytoplasmic membrane where peptidoglycan synthesis occurs. Therefore, a higher concentration of vancomycin is needed to saturate the murein monomers, which are produced at high rates [31].

Vancomycin resistance mediated by the *van* genes was first described in enterococci. The most important genes in staphylococci are the *vanA* and *vanB* genes [32]. The mechanism whereby by the *van* genes mediate resistance consists of a change in the terminal peptide from D-alanyl-D-alanine to D-alanyl-D-lactate, which only occurs after exposure to low vancomycin concentrations. The new dipeptide has low affinity for the drug [33].

4.2 Lipoglycopeptides

Telavancin was recently approved for the treatment of complicated skin and skin structure infections caused by Gram-positive organisms, including *S. aureus* [9]. This antibiotic acts simultaneously on cell-wall synthesis and by disrupting the barrier function of the plasma membrane. The mechanism of cell-wall synthesis inhibition is similar to that of vancomycin, in which the drug binds to the terminal acyl-D-alanyl-D-alanine chains of the cell wall. The second mechanism of action of telavancin, which is believed to be responsible for the destruction of strains with intermediate resistance and heteroresistance to vancomycin, is the interaction of the drug with the cell-wall precursor (lipid II), causing depolarization and increased permeability of the plasma membrane [34].

A surveillance study evaluating the susceptibility of a large collection of Gram-positive strains reported 100% susceptibility to telavancin for MSSA, MRSA and CoNS [35]. The maximum MIC values were 0.5 mg/L for *S. aureus* and 1.0 mg/L for CoNS. In the same study, telavancin was at least 2-fold more potent than other comparable antimicrobial agents when tested against staphylococci, including MRSA [35]. The breakpoints and susceptibility tests for telavancin were approved by the FDA and CLSI in 2014.

Oritavancin is another recently approved lipoglycopeptide, which has a broad spectrum of activity against Gram-positive pathogens, including vancomycin non-susceptible strains [36]. Initial surveillance studies involving 12 countries demonstrated potent *in vitro* activity of oritavancin against MRSA and staphylococci in general [36]. A single dose of oritavancin was not inferior to a regimen with vancomycin consisting of two daily doses administered for 7 to 10 days to treat adults with ABSSSI [37;38].

In contrast to most drugs, oritavancin MICs seem to be higher in *S. aureus* when compared to CoNS (maximum MICs of 4 and 1 mg/L, respectively) [39;40]. Nevertheless, the *S. aureus* isolates were 16-, 8-, 8-, and 4-fold more susceptible to oritavancin than to linezolid, vancomycin, teicoplanin and daptomycin, respectively, and CoNS were 2- to 4-fold more susceptible to oritavancin than to comparable antibiotics [39].

4.3 Lipopeptide

Daptomycin is a lipopeptide that acts on the plasma membrane through a complex process, which culminates in membrane depolarization and permeabilization, with subsequent ion release and cell death [41]. Daptomycin is

recommended by the Infectious Diseases Society of America (IDSA) for the treatment of uncomplicated MRSA bacteremia. A combination of high doses with gentamicin, rifampicin, linezolid, trimethoprim/sulfamethoxazole or a beta-lactam is indicated for complicated MRSA bacteremia or cases that do not respond to vancomycin treatment [42].

Daptomycin resistance was demonstrated in the laboratory by serial passage, and genomic studies have shown that non-susceptible strains carried mutations in the *Mpr*F gene, a lysophosphatidylglycerol synthetase [43]. In CoNS, daptomycin is highly efficient, although reports of resistant strains have been published. Sader et al, [44] found an isolate with a daptomycin MIC of 4 μg/mL, and four resistant strains were identified in a subsequent study [45].

4.4 Oxazolidinones

The mechanism of action of the oxazolidinone linezolid is directed at the early steps of bacterial protein synthesis, in which the drug binds to and reversibly blocks the ribosomal peptidyl transferase center (PTC) [46]. Linezolid was introduced in medical practice as the first anti-MRSA antimicrobial drug after the introduction of vancomycin. Although resistance to linezolid is uncommon among staphylococci, about 2% of isolates present this susceptibility phenotype [47]. In CoNS, the prevalence of resistance ranges from 1% to 3% and resistance can emerge after a short period of treatment, in contrast to *S. aureus* in which linezolid resistance occurs months after the use of the drug [47]. In a recent surveillance study, two *S. aureus* strains and three *S. epidermidis* strains were found to be linezolid resistant among thousands of isolates. The two *S. aureus* strains carried the *cfr* gene [48].

Linezolid resistance in staphylococci is mediated by different mechanisms. One mechanism is modification of the ribosomal PTC through mutations in domain V of the 23S rRNA [46; 49]. The most common mutation includes G2576U [47], but other mutations have been described (G2447U, C2461U, U2500A, G2534U, G2603U, and U2504A) [47].The mutations in the 23S rRNA are directly associated with the linezolid dose and the number of mutated rRNA copies is proportional to the linezolid MIC [50]. Acquisition of the *cfr* gene with methyltransferase activity can induce resistance by modification of A2503 of the 23S rRNA domain V, preventing linezolid and other antimicrobials from binding to the ribosome [51;52]. Isolates carrying *cfr* can be either phenotypically resistant or susceptible [53]. Linezolid resistance can also be associated with mutations in ribosomal proteins L3 and L4 [54].

Tedizolid was approved by the FDA in June 2014 for the treatment of ABSSSI caused by some susceptible bacteria, including *S. aureus* (MRSA and MSSA) [9]. Tedizolid is an oxazolidinone that is 4- to 16-fold more potent against staphylococci than linezolid [55]. The evaluation of tedizolid susceptibility in almost 7,000 isolates of different bacterial species from the United States and Europe, including 80% staphylococci, revealed tedizolid susceptibility in more than 99% of the strains (MICs > 0.5 μg/mL, which is the breakpoint proposed). The modifications associated with resistance were mutations in the genes encoding the 23S rRNA (primarily G2576T), mutations in the ribosomal protein gene L3 or L4, and presence of the multidrug-resistance gene *cfr* [56].

4.5 Streptogramines

The parenteral combination of quinupristin/dalfopristin (Q/D) consists of a group B streptogramin (quinupristin) and a group A streptogramin (dalfopristin) at a ratio of 3:7 [57]. This combination has a proven synergistic *in vitro* activity against staphylococci and other Gram-positive bacteria [58] and is an established alternative to vancomycin to treat MRSA infections [59].Q/D sequentially binds to the 50S ribosomal subunit, inhibiting bacterial protein synthesis [60].

Studies conducted in the last decade have shown 97% susceptibility of *S. aureus* to Q/D [60], and its bacteriostatic activity was not affected by methicillin or quinolone resistance. A recent Chinese study revealed Q/D resistance in only 0.2% of *S. aureus* [59]. The global rates of Q/D resistance range from zero to 3% in different countries. However, alarming resistance rates of 31% and 87% in MRSA have been reported in Taiwan and Northern India, respectively, although Q/D is not used in clinical practice in these countries [61; 62].

The Q/D resistance mechanisms include enzymatic modification of the antibiotic, active transport or efflux mediated by ATP-binding proteins, and alteration of the target site [63]. In staphylococci, the genes associated with streptogramin B resistance include the *erm* genes (*ermA*, *ermB* and *ermC*) that encode 23S rRNA methylation enzymes, the *vgb* genes (*vgbA* and *vgbB*) that encode an antibiotic-inactivating enzyme, and the *msr* genes (*msrA* and *msrB*) that confer streptogramin B resistance by erythromycin-induced active transport. In addition to the streptogramin B resistance genes, the organism needs to carry at least one streptogramin A resistance gene (*vat* or *vga*) for full resistance to Q/D [63]. These include the *vat* genes (*vatA*, *vatB* and *vatC*), which encode acetyltransferases that inactivate streptogramin A, and the *vga* genes (*vgaA*, *vgaB* and *vgaAv*), which encode ATP-binding proteins involved in the active transport of the antibiotic [63]. A mutation in ribosomal protein L22 has also been associated with Q/D resistance in an *S. aureus* strain [64].

4.6 Fluoroquinolones

Moxifloxacin and delafloxacin are fourth-generation quinolones, with the former exhibiting antimicrobial activity against a wide range of bacteria including staphylococci [65]. Moxifloxacin exerts increased activity against Gram-positive cocci, including ciprofloxacin-resistant *S. aureus*, with MICs that are 4- to 64-fold lower than that of

ciprofloxacin [66]. However, despite the good efficacy of moxifloxacin, the presence of resistant *S. epidermidis* isolates has been reported recently by Drew and Paulus [67], and resistance rates have already reached 30.9% among staphylococci according to another study [68].

Whereas most quinolones have higher affinity for DNA gyrase in Gram-negative bacteria and for topoisomerase IV in Gram-positive bacteria, delafloxacin is equally potent against both DNA gyrase and topoisomerase IV [69]. This dual affinity may prevent the selection of resistant mutant populations since at least one mutation in either enzyme gene is needed to significantly reduce susceptibility to that drug [70; 69]. The efflux pumps associated with quinolone resistance, such as NorA, B and C, do not affect delafloxacin susceptibility *in vitro* (Y. Ding and D. Hooper, Massachusetts General Hospital, Boston, MA, USA, personal communication). Delafloxacin is active against *S. aureus*, MRSA, even quinolone-resistant strains, and against multidrug-resistant strains [71; 72]. Compared to other quinolones such as moxifloxacin, delafloxacin shows comparable efficacy and a lower rate of side effects, in addition to acting more efficiently in acid medium [73;74].

4.7 DHFR inhibitors

Iclaprim belongs to the class of selective inhibitors of dihydrofolate reductase (DHFR).The safety and efficacy of the drug have been clinically proven for more than four decades [75]. Trimethoprim is a well-established DHFR inhibitor and has been used both as monotherapy and in combination with other agents (e.g., sulfamethoxazole) [75; 76]. However, despite its good efficacy and safety, the bactericidal activity of trimethoprim is low and resistance has emerged due to the presence of specific mutations in bacterial DHFR [76].

The antibiotic Iclaprim is the result of a drug optimization program and is selective and potent in inhibiting DHFR. This drug is able to inhibit bacterial DHFR with little or no inhibition of the human enzyme [77; 76]. Iclaprim exerts fast *in vitro* bactericidal activity, providing 99.9% of reduction in bacterial load within 6 hours and a post-therapy effect of several hours against MRSA and VISA [77; 78]. Studies have shown that Iclaprim is active against a wide variety of pathogens from different countries and is more potent than trimethoprim against MRSA, VISA, VRSA and other multidrug-resistant staphylococci [79; 76; 77].

4.8 Glycylcyclines

Tigecycline is a semi-synthetic derivate of monocycline and was the first glycylcycline licensed for clinical use [78]. Tigecycline was considered a promising agent with a broad spectrum of activity against a variety of Gram-positive and Gram-negative organisms, including multidrug-resistant staphylococci. However, the clinical use of tigecycline was later associated with a high rate of mortality when administrated to certain patients and is indication was therefore restricted [79].

4.9 Other classes

Ceftaroline is an advanced-generation cephalosporin approved for use to treat pneumonia and acute skin infections caused by bacteria, including staphylococci [80]. Despite good efficacy in the treatment of *S. aureus* infections, a recent study revealed reduced susceptibility to ceftaroline in 2.4% of clinical MRSA isolates [81]. Nevertheless, ceftaroline has shown significant activity against MRSA with reduced susceptibility to vancomycin, daptomycin, clindamycin, levofloxacin, and trimethoprim/sulfamethoxazole [22], and is 16-, 4-8- and 4-fold more active *in vitro* against MSSA than ceftriaxone, linezolid and vancomycin, respectively.

Strains presenting intermediate resistance to ceftaroline were described in Europe and Asia, even before introduction of the drug [82; 83]. Mutations in the *mec*A gene were indicated as being responsible for the reduced antimicrobial activity of ceftaroline [82; 83]. Other mutations, such as $N_{146}K$ and $E_{150}K$, located in non-beta-lactam-binding [82; 83], but directly implicated in the reduced susceptibility to beta-lactams due to modified protein-protein interactions [83], are related to the non-susceptibility to ceftaroline. Mutations at position G239L have also been described in strains with MICs of 2 μg/mL, while isolates exhibiting MICs of 8 μg/mL carried additional alterations in the beta-lactam-binding domain (G447L) [82].

Ceftriaxone, an intravenous long-acting cephalosporin, has shown high efficacy in the treatment of MSSA infections in general [84]. The drug has been widely used due to its long pharmacological half-life and daily dosing. On the other hand, the clinical efficacy of ceftriaxone is a matter of concern because of extensive protein binding of the agent and reduced concentration of the unbound drug [85].

5. Biofilm and resistance

The biofilm of staphylococci, one of their main virulence factors, consists of bacterial aggregates embedded in an extracellular polysaccharide matrix, which facilitates the adherence of microorganisms. The presence of a biofilm impairs the treatment of associated infections. Despite the presence of *in vitro* susceptibility, the difficulty in eradicating biofilms formed on medical devices and the protection that they confer to microorganisms impair the action of

antimicrobial drugs [86]. Furthermore, the biofilm environment favors the transfer of genes among strains, including resistance genes. Studies have suggested an association between genes related to biofilm production and resistance to antimicrobial drugs, especially methicillin and vancomycin [26; 87].

6. Molecular epidemiology of MRSA

Despite advances in treatment options and patient care, infections caused by staphylococci continue to be associated with considerable rates of morbidity and mortality in the hospital and in the community [88]. The clinical and molecular epidemiology of staphylococcal infections has undergone drastic changes in the last two decades, a fact that is attributed to the consistent emergence of MRSA [89]. These changes have been rapidly identified due to the uncommon combination between MRSA strains and low resistance rates to non-beta-lactam antibiotics. Curiously, MRSA clones have emerged and spread among patients who lack the classical risk factors for infection with the resistant microorganism [90]. This phenomenon shows that an MRSA epidemic could originate from an unpredicted area or population. This worrying observation has shed new light on the importance of epidemiological surveillance and the characterization of staphylococcal infections.

In North America and in Europe, this type of surveillance has been extensively conducted in order to permit the identification of new clones of community-acquired MRSA (CA-MRSA) and, more recently, livestock-associated MRSA (LA-MRSA) clones. Although these regions account for less than one-third of the world population, even less is known about the epidemiology of *S. aureus* in other parts of the world. Considering the increased population mobility, there is an urgent need for a better understanding of the epidemiology of *S. aureus* in non-Western areas [89].

Nasal colonization with MRSA is known to be the main cause of both nosocomial and community-acquired infections. Colonization is the carriage of bacteria in the absence of clinical signs or any immunological interaction. Colonizing strains can serve as an endogenous reservoir for subsequent clinical infections or spread in the hospital through indirect transmission (patient-patient), through direct transmission (healthcare worker-patient), or even through contaminated surfaces [91]. According to previous estimates, approximately 20-30% of the world population persistently carries *S. aureus* in their nostrils, while 50% were never colonized [92].

Humans are the natural habitat for many bacteria of the genus *Staphylococcus*, which comprises more than 40 species. The preferred ecological niches in humans include the skin, hair and mucous membranes that line body surface openings. The host immune system or any underlying disease, as well as previous contact with the hospital environment, has been shown to increase antimicrobial resistance in the nasal flora. Colonization rates with methicillin-resistant isolates are usually lower in the community and tend to increase in the hospital. Additionally, these rates are higher among CoNS than among *S. aureus*. This fact is one of the reasons why CoNS are indicated as reservoirs of methicillin resistance [93]

The SCC*mec* elements play an important role in the epidemiology of *S. aureus*. Eleven types and their variants have been described (www.sccmec.org). In general, hospital-acquired MRSA (HA-MRSA) carry SCC*mec* types I-III, while type IV is often found in CA-MRSA, in addition to types VI, VII and IX. SCC*mec* V is carried by LA-MRSA ST398. As reported earlier in this chapter, a novel SCC*mec*, type XI, has been described recently in MRSA isolated from bovine and human samples [94].

There is still a lack of information about the epidemiology of MRSA in health services, especially community health services. This deficit explains the considerable interest in the screening, identification and understanding of MRSA diversity in different scenarios. At present, the molecular techniques most commonly used for the investigation of the molecular epidemiology of these bacteria are protein A gene typing (spa-typing) and multilocus sequence typing (MLST).

Studies using MLST have demonstrated that small sets of strains, called clonal complexes (CC) 5, CC8, CC22, CC30 and CC45, are associated with HA-MRSA infections. Furthermore, studies have shown that the geographically distinct strains CC1, CC8, CC30 and CC80 are associated with the occurrence of CA-MRSA infections, while CC8 and CC30 have been identified as pandemic lineages associated with both environments. Regional clones have also been described in Australia (ST93), India (ST772), South Korea (ST72), and Taiwan and China (ST59) [95].

Extensive dissemination of HA-MRSA clones in the community has been observed in recent years. Although the frequency of MRSA is decreasing in countries such as Belgium, France, Germany, the United Kingdom and Portugal, the prevalence of MRSA is still relatively high [96]. However, the transmission of different MRSA clones between the hospital and community makes the use of a dichotomous key for the classification of strains [94]. MRSA strains acquired in these two environments have distinct phenotypic and genotypic characteristics. While HA-MRSA carries a larger and multidrug-resistant SCC*mec*, CA-MRSA possess a smaller SCC*mec* element with a limited resistance profile. The hospital lineages also carry type IV, as do the epidemic clone EMRSA-15 and some pediatric clones [96; 97]. CA-MRSA clones often harbor specific virulence genes, such as the genes encoding Panton-Valentine leukocidin (lukF-PV and lukS-PV), the arginine catabolic mobile element (ACME), and other highly expressed toxins [97].

All over the world, CA-MRSA are associated with specific genetic lineages such as USA300, USA400, ST30-IV (South Pacific West clone), ST59-V (Taiwan clone), and ST80-IV (European clone) [96]. USA300, the predominant clone found in North America, is associated with the occurrence of most community-associated skin and soft-tissue

infections, and is responsible for an increase in the proportion of healthcare-associated bloodstream infections. As mentioned earlier, MRSA strains have emerged in animals, particularly livestock. The predominant LA-MRSA strain belongs to the ST398 lineage, a pig-associated clone that was also isolated from infections in calves, domestic birds and humans [98].

There is evidence in different countries that previously described resistant clones are replaced with novel clonal types. However, this trend is unknown in most parts of the world. Nevertheless, information about changes in clonal identities and their geographic dissemination is important to understand the spread and evolution of MRSA [94].

7. Epidemiology of CoNS

Historically, few CoNS strains have been identified to species level, including *S. saprophyticus*, *S. epidermidis*, *S. haemolyticus* and *S. lugdunensis*, while others receive the generic denomination of CoNS [99]. A large proportion of hospital isolates of CoNS is resistant to most antibiotics available in clinical practice. The origin of infections caused by these bacteria was initially considered endogenous. However, studies conducted over the last decades have shown that some hospital CoNS genotypes are opportunistic pathogens in healthcare-associated infections [100].

Different typing methods have been used in studies on CoNS. The typing systems can be based on phenotypic or genotypic criteria. The phenotypic methods include biochemical reactivity, antimicrobial susceptibility testing, serological typing, phage typing, biofilm production, and analysis of plasmid or protein profiles. However, these methods exhibit low discriminatory power for closely related strains. More discriminatory genotypic methods are pulsed-field gel electrophoresis (PFGE) combined with MLST, DNA sequencing and, more recently, MALDI-TOF mass spectrometry, a soft ionization technique that analyzes protein patterns directly detected in the microorganisms [101].

Analysis of the molecular epidemiology of *S. epidermidis* has demonstrated an extensive genetic diversity among isolates. However, most of these studies have detected MRSE clusters that can persist in the hospital for many years. Furthermore, indistinguishable genotypes of *S. epidermidis* have been identified in samples isolated from different nurseries and hospitals, indicating the specific dissemination of some lineages among different settings [100].
Staphylococcus haemolyticus is an emerging cause of hospital infections, which is mainly associated with infections in newborns and immunocompromised patients. This species is the second most common CoNS isolated from blood cultures, after *S. epidermidis. Staphylococcus haemolyticus* is highly resistant to commonly used antimicrobial agents and seems to acquire resistance elements easily [102].

Another CoNS species associated with the occurrence of urinary tract infections, *S. saprophyticus*, has been a matter of great concern [100]. The pathogenesis suggested for this type of infection is similar to that of *E. coli*. However, little is known about the epidemiology of *S. saprophyticus* and the involvement of specific strains or clones in urinary tract infections.

In addition to these species, *S. lugdunensis* has gained notoriety as a cause of Gram-positive infections. This species can cause severe infections similar to those caused by *S. aureus*, such as catheter-associated bacteremias, septicemia, endocarditis, vascular aneurysms, and osteomyelitis. Notably, skin disruption in the inguinal region (vascular access cases) is associated with invasive infections by *S. lugdunensis*, especially in patients with kidney problems [99].

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